

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
3 February 2005 (03.02.2005)

PCT

(10) International Publication Number
WO 2005/010034 A1

(51) International Patent Classification⁷: **C07K 14/165**,
A61K 39/215, 39/42, 38/16

(21) International Application Number:
PCT/US2004/023345

(22) International Filing Date: 20 July 2004 (20.07.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/489,166 21 July 2003 (21.07.2003) US
60/524,642 25 November 2003 (25.11.2003) US

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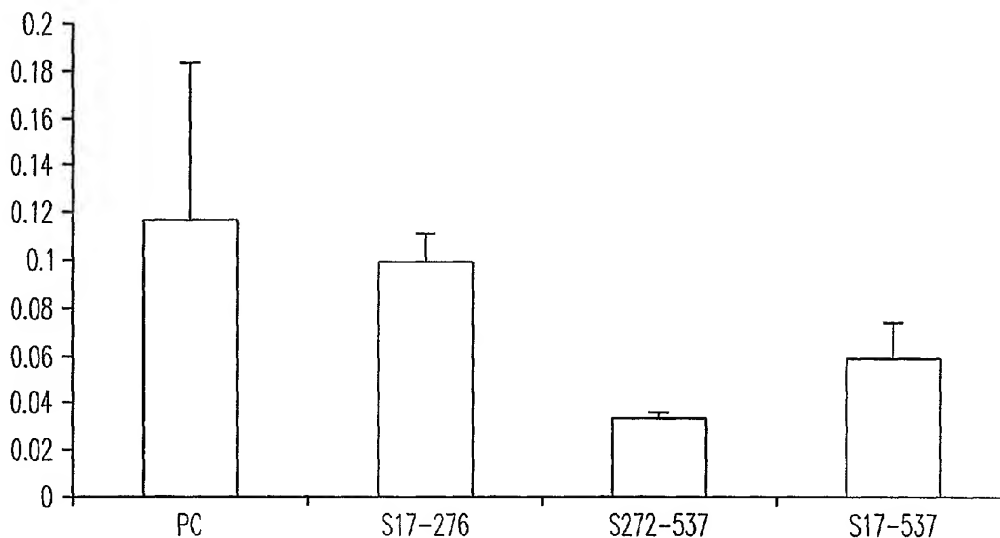
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(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
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KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
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TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),

[Continued on next page]

(54) Title: SOLUBLE FRAGMENTS OF THE SARS-COV SPIKE GLYCOPROTEIN



(57) Abstract: The invention relates generally to the spike protein from the virus (SARS-CoV) that is etiologically linked to severe acute respiratory syndrome (SARS); polypeptides and peptide fragments of the spike protein, and conservative variants thereof; nucleic acid segments and constructs that encode the spike protein, polypeptides and peptide fragments of the spike protein, and conservative variants thereof, and coupled proteins that include the spike protein or a portion thereof; peptidomimetics; vaccines; methods for vaccination and treatment of severe acute respiratory syndrome; antibodies; aptamers; and kits containing antibodies or aptamers that bind to the spike protein.

WO 2005/010034 A1



European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IIU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Soluble Fragments of the SARS-CoV Spike Glycoprotein

This application claims priority from U.S. Application Ser. No.
5 60/489,166 filed July 21, 2003 and from U.S. Application Ser. No. 60/524,642
filed November 25, 2003, which are hereby incorporated by reference in their
entireties.

Government Funding

The invention described herein was developed with the support of the
10 Department of Health and Human Services. The United States Government has
certain rights in the invention.

Field of the Invention

The invention relates generally to a spike polypeptide that is encoded by
15 a coronavirus (herein SARS-CoV), which is etiologically linked to Severe Acute
Respiratory Syndrome (SARS). The invention further relates to nucleic acids
and polypeptides having amino acid sequences that correspond to fragments of
spike protein of SARS-CoV, and conservative variants thereof. The invention
also relates to use of these nucleic acids, polypeptides, variants, and fragments to
20 produce antibodies that recognize the spike protein of SARS-CoV, and for the
production of vaccines against SARS. Another aspect of the invention relates to
spike protein fragments for inhibiting fusion of the SARS-CoV with animal
cells.

Background of the Invention

25 Severe acute respiratory syndrome (SARS) is an infectious atypical
pneumonia that has recently been recognized in patients in 32 countries and
regions. The atypical pneumonia with unknown etiology was initially observed
in Guangdong Province, China. This observation was followed by reports from
Hong Kong, Vietnam, Singapore, Canada and Beijing of severe febrile
30 respiratory illness that spread to household members and health care workers.
This disease was later designated "severe acute respiratory syndrome (SARS)"
by the World Health Organization (WHO). Until May 19, 2003, a cumulative

total of 7,864 SARS cases were reported to WHO from 29 countries. A total of 643 deaths (case-fatality proportion: 8.2 %) were reported.

5 Researchers around the world have sequenced the genome of SARS causing viruses from different regions of the globe. The viruses have been classified as coronaviruses. Coronaviruses have been grouped into three categories based on cross-reactivity of antibodies backed up by genetic data. Two previously known human viruses fell into different groups than SARS-CoV. The coronavirus that causes SARS does not fit into any of the previously known clusters. Rather, it forms a new group by itself. Phylogenetic analysis of
10 the predicted viral proteins indicates that the virus does not closely resemble any of the three previously known groups of coronaviruses. Most coronaviruses cause either a respiratory or an enteric disease, which is also transmitted by the faecal-oral route.

The incubation period for SARS is usually 2 to 7 days. Infection is
15 characterized by fever, non-productive cough, shortness of breath, and the presence of minimal auscultatory findings with consolidation on chest radiographs. Lymphopenia, leucopenia, thrombocytopenia, and elevated liver enzymes and creatinine kinase may also be present in most cases. Symptoms relating to the gastrointestinal tract were also noticed in SARS patients.

20 Pathological studies of patients who died of SARS from Guangdong, Hongkong, Beijing and Singapore showed diffuse alveolar damage (DAD) in the lung as the most notable feature. In those individuals with severe disease resulting in death, scattered type II pneumocytes showed marked cytologic changes that include multinucleation, cytomegaly, nucleomegaly, clearing of
25 nuclear chromatin, and prominent nucleoli. Although these changes were severe, they were within the spectrum of epithelial changes seen in other cases of diffuse alveolar damage. Morphologic changes that were identified included bronchial epithelial denudation, loss of cilia, and squamous metaplasia. Other findings included focal intraalveolar hemorrhage, hemophagocytosis, necrotic
30 inflammatory debris in small airways, organizing pneumonia or secondary bacterial pneumonia.

The pathogenesis of this disorder remains to be determined. However, the mechanism of acute lung injury could involve direct damage by the virus to the alveolar wall by targeting either endothelial cells or epithelial cells.

Alternatively, the virus could infect inflammatory cells with the injury mediated through cytokines, interleukins, or tumor necrosis factor-alpha. It is also possible that the tissue damage in SARS is not directly related to viral infection in tissues but is a secondary effect of cytokines or other factors induced by viral infection proximal to but not within the lung tissue.

Pathologic evaluation of the fatal cases showed that hepatocytes underwent fatty degeneration, cloudy swelling, apoptosis and dot necrosis, with Kupffer cell proliferation and portal infiltrates of lymphocytes. There were regional hemorrhages, vascular congestion and lymphocytic infiltration in gastrointestinal walls of the patient.

Due to the ability of SARS-CoV to be spread through an airborne route, SARS-CoV presents a particular threat to the health of large populations of people throughout the world. Accordingly, methods to immunize people before infection, diagnose infection, immunize people during infection, and treat infected persons infected with SARS-CoV are greatly needed.

Summary of the Invention

These and other needs are met by the invention described herein. The invention provides polypeptides; peptide fragments; viral fusion inhibitors; coupled proteins; immunopeptides; immune compositions; peptidomimetics; nucleic acid segments; expression cassettes; nucleic acid constructs; recombinant viruses; viral vaccines; peptide vaccines; microorganism vaccines; DNA vaccines; antibodies; aptamers; pharmaceutical compositions; methods to immunize an animal; a method to treat severe acute respiratory syndrome (SARS); methods to diagnose SARS; and kits.

The invention provides polypeptides having an amino acid sequence corresponding to that of a polypeptide that is etiologically linked to SARS. Preferably the polypeptide is the spike protein from SARS-CoV that can inhibit SARS fusion with animal cells and/or raise immune response in an animal. In some embodiments, the polypeptide is a soluble form of the spike protein from SARS-CoV. In other embodiments, the polypeptide includes amino acids 17-757 of the spike protein from SARS-CoV. In some embodiments, the polypeptide includes amino acids 762-1189 of the spike protein from SARS-CoV. In other embodiments, the polypeptide includes amino acids 17-757 of the

spike protein from SARS-CoV. In some embodiments, the polypeptide includes amino acids 17-276 of the spike protein from SARS-CoV. In other embodiments, the polypeptide includes amino acids 303-537 of the spike protein from SARS-CoV. In some embodiments, the polypeptide includes amino acids 317-517 of the spike protein from SARS-CoV. In other embodiments, the polypeptide includes amino acids 272-537 of the spike protein from SARS-CoV. In some embodiments, the polypeptide includes amino acids 17-537 of the spike protein from SARS-CoV. In other embodiments, the polypeptide includes amino acids 17-1189 (relative to SEQ ID NO: 1) of the spike protein from SARS-CoV. The polypeptides of the invention can inhibit SARS-CoV fusion with animal cells. The nucleic acids and polypeptides of the invention can elicit an immune response when used to inoculate an animal. In some embodiments, the nucleic acids and polypeptides of the invention elicit a cellular immune response when used to inoculate an animal. In other embodiments, the nucleic acids and polypeptides of the invention elicit a humoral immune response when used to inoculate an animal. The animal can be a reptile. In some embodiments, the animal is an avian. In other embodiments, the animal is a mammal. Sometimes, the animal is a human.

The invention provides peptide fragments of the spike protein from SARS-CoV. Preferably the peptide fragments are soluble in aqueous solution. A peptide fragment of the invention may lack one amino acid residue from the amino acid sequence of the full length spike protein from SARS-CoV. In some embodiments, peptide fragments are at least three amino acids in length. In other embodiments, peptide fragments are at least 10 amino acids in length. In some embodiments, peptide fragments are at least 20 amino acids in length. In other embodiments, peptide fragments are at least 30 amino acids in length. In some embodiments, peptide fragments are at least 40 amino acids in length. In other embodiments, peptide fragments are at least 50 amino acids in length. In some embodiments, peptide fragments are at least 60 amino acids in length. The peptide fragments may also be single amino acid unit additions to a fragment of a given length. For example, peptide fragment may be 3, 4, 10, 11, 21, 22, 31, or 32 amino acids in length. The peptide fragments of the invention can inhibit SARS Co-V fusion with animal cells or elicit an immune response when used to inoculate an animal. Examples of peptides that can elicit an immune response

after inoculation of an animal include, for example, the D24 peptide having sequence DVQAPNYTQHTSSMRGC (SEQ ID NO:58) and the P540 peptide having sequence PSSKRFQPQQFGRDC (SEQ ID NO:59). In some embodiments, the peptide fragments of the invention elicit a cellular immune response when used to inoculate an animal. In other embodiments, the peptide fragments of the invention elicit a humoral immune response when used to inoculate an animal. The animal can be a reptile. In some embodiments, the animal is an avian. In other embodiments, the animal is a mammal. In further embodiments, the animal is a human.

10 The invention provides coupled proteins. The coupled proteins include a carrier protein that is coupled to a second polypeptide. Preferably, the carrier protein is soluble. In some embodiments, the carrier protein increases an immune response to the second polypeptide of the coupled protein when used to inoculate an animal. In other embodiments, the carrier protein elicits a cellular immune response to the second polypeptide of the coupled protein when used to inoculate an animal. In some embodiments, the carrier protein elicits a humoral immune response to the second polypeptide of the coupled protein when used to inoculate an animal. The second polypeptide can be a polypeptide or a peptide fragment of the invention, or a conservative variant thereof. The animal can be a reptile. In some embodiments, the animal is an avian. In other embodiments, the animal is a mammal. In further embodiments, the animal is a human.

25 The invention provides immunopeptides that include a polypeptide or peptide fragment of the invention, or a conservative variant thereof, that is coupled to an acetyl group, a picryl group, an arsanilic acid, or to a sulfanilic acid. In some embodiments, the immunopeptide is coupled to an acetyl or a picryl group. In other embodiments, immunopeptide is coupled to arsanilic acid or sulfanilic acid. Preferably, the immunopeptide is soluble. Preferably, the immunopeptide elicits an immune response when used to inoculate an animal. In some embodiments, the immunopeptide elicits a humoral immune response when used to inoculate an animal. In other embodiments, the immunopeptide elicits a cellular immune response when used to inoculate an animal. The animal can be a reptile. In some embodiments, the animal is an avian. In other embodiments, the animal is a mammal. In further embodiments, the animal is a human.

The invention provides peptidomimetics that are polypeptides or peptide fragments of the invention, and conservative variants thereof, in which a peptide bond has been replaced with a non-peptide bond. In some embodiments, the peptidomimetic can inhibit SARS Co-V fusion with animal cells. In other
5 embodiments, the peptidomimetic elicits an immune response when used to inoculate an animal. For example, the peptidomimetic can elicit a cellular immune response when used to inoculate an animal. Alternatively, the peptidomimetic elicits a humoral immune response when used to inoculate an animal. The animal can be a reptile. In some embodiments, the animal is an
10 avian. In other embodiments, the animal is a mammal. In further embodiments, the animal is a human.

The invention provides compositions containing an adjuvant and a nucleic acid, polypeptide, a peptide fragment, or a peptidomimetic of the invention. In some embodiments, the composition inhibits SARS-CoV fusion
15 with animal cells. In other embodiments, the composition elicits an immune response when used to inoculate an animal. In some embodiments, the immune composition elicits a cellular immune response when used to inoculate an animal. In other embodiments, the immune composition elicits a humoral immune response when used to inoculate an animal. The animal can be a reptile.
20 In some embodiments, the animal is an avian. In other embodiments, the animal is a mammal. In further embodiments, the animal is a human.

The invention provides nucleic acid segments that encode polypeptides and peptide fragments of the invention, and conservative variants thereof.

The invention provides expression cassettes having a promoter that is
25 operably linked to a nucleic acid segment of the invention. In some embodiments, the promoter is constitutive. In other embodiments, the promoter is inducible.

The invention provides nucleic acid constructs that include a vector and a nucleic acid segment of the invention. The nucleic acid construct can include an
30 expression cassette of the invention. In some embodiments, the vector can be a virus. In other embodiments, the vector is a plasmid. In further embodiments, the vector is an expression vector.

The invention provides a recombinant virus that includes a viral vector and a nucleic acid segment of the invention. In some embodiments, the viral

vector is a herpes virus. In other embodiments, the viral vector is a canarypox virus. In other embodiments, the viral vector is an adenovirus. In further embodiments, the viral vector is a vaccinia virus.

The invention provides a viral vaccine against SARS that includes a viral
5 vector, a nucleic acid segment of the invention, and a pharmaceutical carrier. In some embodiments, the viral vector is a herpes virus. In other embodiments, the viral vector is a canarypox virus. In other embodiments, the viral vector is an adenovirus. In further embodiments, the viral vector is a vaccinia virus. Preferably, the pharmaceutical carrier is formulated for injection. Preferably, the
10 viral vaccine elicits an immune response when used to inoculate an animal. In some embodiments, the viral vaccine elicits a cellular immune response when used to inoculate an animal. In other embodiments, the viral vaccine elicits a humoral immune response when used to inoculate an animal. The animal can be a reptile. In some embodiments, the animal is an avian. In other embodiments,
15 the animal is a mammal. In further embodiments, the animal is a human.

The invention provides a peptide vaccine against SARS that includes a peptidomimetic, polypeptide or a peptide fragment of the invention, or a conservative variant thereof, and a pharmaceutical carrier. Preferably, the pharmaceutical carrier is formulated for injection. Preferably, the peptide
20 vaccine is formulated in unit dosage form. Preferably, the peptide vaccine elicits an immune response when used to inoculate an animal. In some embodiments, the peptide vaccine elicits a cellular immune response when used to inoculate an animal. In other embodiments, the peptide vaccine elicits a humoral immune response when used to inoculate an animal. The animal can be a reptile. In
25 some embodiments, the animal is an avian. In other embodiments, the animal is a mammal. In further embodiments, the animal is a human.

The invention provides a microorganism vaccine against SARS that includes a microorganism that expresses a polypeptide or a peptide fragment of the invention, or a conservative variant thereof, and a pharmaceutical carrier.
30 Preferably, the microorganism is attenuated. In some embodiments, the microorganism is Salmonella. In other embodiments, the microorganism is Listeria. In further embodiments, the microorganism is Listeria monocytogenes. In some embodiments, the pharmaceutical carrier is formulated for injection. In other embodiments, the pharmaceutical carrier is formulated for oral

administration. Preferably, the microorganism vaccine is formulated in unit dosage form. Preferably, the microorganism vaccine elicits an immune response when used to inoculate an animal. In some embodiments, the microorganism vaccine elicits a cellular immune response when used to inoculate an animal. In
5 other embodiments, the microorganism vaccine elicits a humoral immune response when used to inoculate an animal. The animal can be a reptile. In some embodiments, the animal is an avian. In other embodiments, the animal is a mammal. In further embodiments, the animal is a human.

The invention provides a DNA vaccine against SARS that includes a
10 vector into which is inserted a nucleic acid segment of the invention, and a pharmaceutical carrier. The DNA vaccine may include an adjuvant. The DNA vaccine may include a myonecrotic agent. For example, the myonecrotic agent can be bupivacaine. In other embodiments, the myonecrotic agent is cardiotoxin. The vector can, for example, be a virus. In other embodiments, the vector is a
15 bacteriophage. In further embodiments, the vector is a plasmid. The vector containing the insert can be prepared in a eukaryotic cell. However, in some embodiments, the vector containing the insert is prepared in a prokaryotic cell. For example, the vector containing the insert can be prepared in a bacterium. In some embodiments, the pharmaceutical carrier is formulated for mucosal
20 delivery. In other embodiments, the pharmaceutical carrier is formulated for injection. Preferably, the DNA vaccine is formulated in unit dosage form. Preferably, the DNA vaccine elicits an immune response when used to inoculate an animal. In some embodiments, the DNA vaccine elicits a humoral immune response when used to inoculate an animal. In other embodiments, the DNA
25 vaccine elicits a cellular immune response when used to inoculate an animal. The animal can be a reptile. In some embodiments, the animal is an avian. In other embodiments, the animal is a mammal. In further embodiments, the animal is a human.

The invention provides an antibody that binds to a polypeptide or peptide
30 fragment of the invention, or a conservative variant thereof. In some embodiments, the antibody is an antigen-binding antibody fragment. In other embodiments, the antibody is a polyclonal antibody. In further embodiments, the antibody is a single-chain antibody. In other embodiments, the antibody is a monoclonal antibody. In some preferred embodiments, the antibody is a

humanized antibody. The antibody may be coupled to a detectable tag. For example, the detectable tag can be a radiolabel. In some embodiments, the detectable tag is an affinity tag. In other embodiments, the detectable tag is an enzyme. In further embodiments, the detectable tag is a fluorescent protein. In some preferred embodiments, the detectable tag is a fluorescent marker. The antibody may also be coupled to a toxin.

The invention provides aptamers that bind to a polypeptide or peptide fragment of the invention, or a conservative variant thereof. The aptamer may be coupled to a detectable tag. For example, the detectable tag is a radiolabel. In some embodiments, the detectable tag is an affinity tag. In other embodiments, the detectable tag is an enzyme. In further embodiments, the detectable tag is a fluorescent protein. In some preferred embodiments, the detectable tag is a fluorescent marker. The aptamer may also be coupled to a toxin.

The invention provides a pharmaceutical composition or a kit containing an antibody, S polypeptide or aptamer of the invention and a pharmaceutical carrier. Preferably, the pharmaceutical composition is formulated for injection.

Brief Description of the Figures

This patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Fig. 1A illustrates an agarose gel electrophoresis of a DNA construct having an insert that encodes the spike protein of the invention. Lanes from left to right: Lane 1 is a one kb DNA ladder (markers from bottom to top – 0.5, 1.0, 1.6, 2.0, 3.0, 4.0); Lane 2 shows the DNA construct digested with BamHI/XbaI, resulting in the distinctive vector band (upper band) and the DNA fragment that encodes the spike protein (lower band); Lane 3 shows the DNA construct digested with HindIII which produced a smaller band and a larger band as expected due to the presence of a HindIII site in the vector and within the DNA fragment encoding the spike protein.

Fig. 1B provides a schematic diagram of a monomer of the full-length SARS-CoV S glycoprotein showing various soluble polypeptide fragments after

removal of the signal sequence (residues 1–16, SEQ ID NO:60). The soluble fragments are spike protein fragments named “S” followed by numbers corresponding to the spike protein amino acids that constitute the termini of the fragment. Thus, “S756” is a soluble spike protein fragment beginning at amino acid 17 (just after the signal sequence) and ending at amino acid 756. “TM” denotes the transmembrane segment and the arrow indicates a possible cleavage site within amino acid positions 758–761 (sequence RNTR). “RBD” indicates the potential receptor-binding domain that is within amino acid positions 272–537 (SEQ ID NO:57), likely between a residue downstream from position 303 and a residue upstream of position 537 (SEQ ID NO:61).

Fig. 2 illustrates a denaturing polyacrylamide gel electrophoresis (SDS-PAGE) of the expression of a peptide fragment of the spike protein from SARS-CoV in *Escherichia coli*. The peptide fragment corresponds to amino acids 17–446 of SEQ ID NO: 1. The nucleic acid segment encoding amino acids 17–446 was cloned into a pRSET vector to create pRSET-S(17–446), which was expressed in BL21DE3 cells. Numbers and arrows on the left indicate molecular weight markers in kilodaltons. The lanes contain the following polypeptides: M - molecular weight markers; lanes 1 and 2 - polypeptides of control *E. coli* containing the pRSET vector without the nucleic acid segment encoding amino acid residues 17–446 of SEQ ID NO: 1 and without isopropylthiogalactoside (IPTG) induction; lane 3 - polypeptides of control *E. coli* containing the pRSET vector without the nucleic acid segment encoding amino acid residues 17–446 of SEQ ID NO: 1 but with IPTG induction; lane 4 - analysis of *E. coli* containing the pRSET vector with a nucleic acid segment encoding amino acid residues 17–446 of SEQ ID NO: 1, and with IPTG induction. The arrow on the right side indicates the position of a peptide fragment corresponding to amino acid residues 17–446 of SEQ ID NO: 1 as expressed in *E. coli*.

Fig. 3 illustrates a slot blot analysis of the expression of the indicated peptide fragments of the spike protein from SARS-CoV in mammalian cells. Nucleic acid segments coding for the peptide fragments were cloned into a pSecTag2B vector to express peptide fragments having the mouse k chain leader sequence at the N-terminus for secretion, and a c-Myc epitope plus a histidine tag at the C-terminus for detection and affinity purification. The nucleic acid constructs were transformed into HEK293 and VeroE6 cells. Expression of the

indicated peptide fragments was examined through use of slot blot analysis with an anti-c-Myc antibody. The numbers on the left and right indicate the amino acid residues included within the detected peptide fragments. The left column represents expression of the peptide fragments in HEK293 cells. The right column represents expression of the peptide fragments in VeroE6 cells. The upper half represents samples obtained from medium in which the cells were grown (secreted proteins), and the lower half represents samples obtained from cell lysate (intracellular portion). PC is a positive control, provided by the manufacturer of the plasmid that contains PSA with a c-Myc tag at the C-terminus. NC is a negative control that contains the full length spike protein from SARS-CoV that lacks a c-Myc epitope or histidine tag.

Fig. 4A illustrates a slot blot analysis of the expression of the indicated peptide fragments from the spike protein from SARS-CoV in human 293 or Monkey VeroE6 cells. Supernatants of 293 and Vero E6 cells transfected with plasmids encoding S fragments (S276, S537, and S756) in the absence or presence of T7 polymerase expressed by recombinant vaccinia virus (VTF7.3) were transferred to nitrocellulose membranes and detected with anti-c-Myc epitope antibody. The numbers on the left and right indicate the amino acid residues included within the detected peptide fragments. PSA PC is a positive control that contains PSA with a c-Myc tag at the C-terminus. pCDNA-S NC is a negative control that contains the full length spike protein from SARS-CoV that lacks a c-Myc epitope or histidine tag. The lanes are as follows: (1) human 293 cells that were not infected with a VTF7.3 vaccinia virus, (2) human 293 cells that were infected with a VTF7.3 vaccinia virus, (3) monkey VeroE6 cells that were not infected with a VTF7.3 vaccinia virus, and (4) monkey VeroE6 cells that were infected with a VTF7.3 vaccinia virus.

Fig. 4B Supernatants from transfected cells as described above for Fig. 4A were incubated with Ni-NTA agarose beads, washed, and subjected to Western blotting with the same anti-c-Myc epitope antibody as in Fig. 4A.

Fig. 4C illustrates detection of S fragments by two rabbit polyclonal antibodies raised against peptides corresponding to sequences starting at residues 24 (D24, middle panel) and 540 (P540, right panel), respectively. The left panel shows for comparison Western blot where S537 and S756 were detected by the anti-c-Myc epitope antibody.

Fig. 5 illustrates that the full-length membrane-associated S protein is expressed on the surface of cells, as shown by flow cytometry using the rabbit polyclonal antibody P540. A nucleic acid encoding the full-length S glycoprotein was used to transfect 293 cells, which were then infected with VTF7.3. Cells were collected and incubated with P540 polyclonal antibody plus anti-rabbit secondary antibody conjugated with FITC, washed, and subjected to flow cytometry analysis. The same plasmid used to express S but without the nucleic acids for S was used to transfect cells in a control experiment denoted as negative control (NC); cells with nucleic acids encoding the full-length S glycoprotein are denoted as S.

Fig. 6A and 6B illustrate that substantially no cleavage of the S glycoprotein occurs naturally. Western blots of supernatants from transfected 293 cells expressing S756, Se, and cell lysate of 293 cells expressing the S glycoproteins using the P540 antibody are shown. Close to background level cleavage of S and Se was observed. Fig. 6A shows a Western blot of samples kept for three days at 4 °C before analysis to monitor the effect of nonspecific protease activity on the cleavage pattern. In contrast, Fig. 6B shows blots with samples used immediately after preparation.

Fig. 7A-C shows that cell fusion is mediated by the S glycoprotein. A pCDNA3-based plasmid without S insert was used as plasmid control, and fusion between S-expressing cells with ACE2-ecto expressing cells was used as negative control. The pCDNA3-ACE2-ecto construct expresses just the ACE2 soluble ecto domain tagged with C9 peptide. Fig. 7A illustrates that there was no syncytium formation between 293T cells transfected with pSecTag2B-S and pCDNA3-ACE2-Ecto. In contrast, Fig. 7B illustrates syncytium formation between 293T cells transfected with pSecTag2B-S and pCDNA3-ACE2, respectively. Fig. 7C graphically illustrates cell fusion as measured by a reporter gene-based assay. As shown, S glycoprotein expressed in both pCDNA3 and pSecTag2B vectors can be detected in a β -gal reporter gene-based cell-cell fusion assay.

Fig. 8A-C shows that the S glycoprotein receptor-binding domain (RBD) is localized between residues 272 and 537. Fig. 8A illustrates binding of two different S soluble fragments (S537 and S756) to 293 and Vero E6 cells. Fig. 8B illustrates binding of various S fragments to Vero E6 cells. The background

OD₄₀₅ measured for the negative control was subtracted from the OD₄₀₅ values of each S fragment. The resulting OD₄₀₅ for each fragment was then presented as a percentage of the OD₄₀₅ for S537. Fig. 8C illustrates which S polypeptide fragments interact with purified soluble ACE2 as measured by ELISA. In all experiments, the negative control (NC) represents sample processed exactly the same way as the others except that the plasmid used for transfection did not encode any protein. Data shown here represent at least three independent experiments. OD₄₀₅ for all samples is presented as percentages of the OD₄₀₅ for S537.

Fig. 9A-D illustrates that dimerization occurs between the N terminal fragments of the SARS-CoV S glycoprotein as demonstrated by co-immunoprecipitation and cross-linking. All N-terminal fragments except the smallest fragment (S317-517) containing the receptor binding domain were coimmunoprecipitated with S756 by the P540 antibody. The P540 antibody is a rabbit polyclonal antibody that was developed against a peptide containing residues 540-555 of the S glycoprotein and it binds the S756 polypeptide but not the N-terminal fragments.

In Fig. 9A, plasmids encoding N-terminal fragments (denoted by the number of the ending amino acid residue or the number of the starting and ending residue) were used to transfect 293T cells alone (left six lanes) or in combination (right four lanes) with a S756-encoding plasmid. These cells were then infected with the vaccinia virus VTF7.3. After incubation, the culture medium was collected and subjected to Western blot analysis using a mouse anti-c-Myc epitope antibody that recognizes all fragments.

Fig. 9B shows that all N-terminal S fragments, except the smallest fragment (S317-517) that contained the receptor binding domain, were coimmunoprecipitated with S756 by the P540 antibody. The same medium samples used in Fig. 9A were subjected first to immunoprecipitation with the P540 polyclonal antibody that recognizes only S756. These immunoprecipitates were then subjected to Western blot analysis using the anti-c-Myc epitope antibody to confirm that the N-terminal fragments coimmunoprecipitated.

Fig. 9C shows that a new band with a molecular weight corresponding to a dimer forms in the presence and absence of DTT. To rule out the possibility of nonspecific disulfide bond formation that may lead to coimmunoprecipitation,

DTT was included in one of the coimmunoprecipitation experiment. DTT had no effect on either immunoprecipitation or coimmunoprecipitation of secreted S756 (left lanes) or S756+S276 (right lanes). Medium samples containing secreted S756 (left lanes) or S756+S276 (right lanes) fragments were subjected to immunoprecipitation with P540 in the presence or absence of 2 mM DTT.

Fig. 9D illustrates the size of the S polypeptide oligomers. The S537 fragment was cross-linked with BS³ (Pierce, Rockford, IL) as described in the Examples and a Western blot was prepared after SDS-PAGE separation and the anti-c-Myc antibody was used for detection of the S537 monomer and its oligomers. As shown in the right lane of Fig. 9D, a new band appears when the crosslinking reagent is added. The new band had a molecular weight corresponding to a dimer but not of higher order oligomers.

Fig. 10A illustrates dimerization of the N terminal fragment S537 as detected by size-exclusion chromatography. The elution profiles of S537 and S317-517 are shown with arrows and numbers indicating the position and molecular weight at which standard calibration proteins were eluted.

Fig. 10B provides western blots of fractions collected for S537 and S317-517 by using an anti-c-Myc epitope antibody.

Fig. 11A-B illustrates that the extreme N terminal domain is required for the S glycoprotein mediated cell-cell fusion. Fig. 11A provides a schematic representation of the S glycoprotein deletion mutants and a summary of the data from a cell-cell fusion assay where RBD denotes the approximate position of the receptor binding domain. The presence of signal due to fusion is denoted by a plus (+) and lack of measurable signal above background levels by a minus (-). Only wild type polypeptides with amino acids 17-1255 had fusion activity. Neither of the deletion mutants having amino acids 103-1255 (Del1) or 311-1255 (Del2) had fusion activity. Fig. 11B shows the levels of expression of full length and deletion mutants of the S glycoprotein as measured by Western analysis. Equal amount of cell lysates were loaded for each sample and the rabbit polyclonal antibody P540 was used for detection. Fig. 11C illustrates that the full length S glycoprotein and the Del1 and Del 2 deletion mutants are expressed on the cell surface as measured by flow cytometry. The level of surface expression was low although the negative control where the cells were

transfected with an empty plasmid was clearly distinguishable to the left of the other three curves.

Fig. 12A-B illustrates that dimeric S1 binds more efficiently to the receptor ACE2 than monovalent fragments containing the receptor binding domain. Fig. 12A shows the relative levels of expression of different S fragments as detected by ELISA using 200 μ l of culture supernatants from cells transfected with S276, S319-518 and S537 constructs. Anti-His and anti-c-Myc epitope antibodies were used in a sandwich ELISA to detect the levels of secreted tagged S proteins. Fig. 12B shows the level of binding by S fragments to ACE2 as measured by ELISA. The tagged ACE2 was bound to plates by an anti-C9 antibody that had been previously coated on the plates. The supernatants from cell cultures where the cells were transfected with various S proteins were mixed and incubated in ELISA plates either with (hatched bars) or without (open bars) anti-c-Myc antibody. The highest level of expression or binding is assumed to be 100 %. As shown the S537 fragment with both the N-terminal dimerization domain and the receptor binding domain, binds ACE2 more efficiently than does the S319-518 fragment that has only the receptor binding domain.

Fig. 13A-B illustrates that the soluble S ectodomain is trimeric under the conditions of size-exclusion chromatography. In Fig. 13A, purified Se was run on a gel filtration column that was calibrated by using proteins with known molecular weight. BSA in equal amount was included as an internal control. In Fig. 13B, different fractions were collected from the gel filtration column and analyzed by Western blot. Two bands S polypeptide are detected in some fractions that contain Se fragments of the indicated molecular weights, representing the Se fragment alone (lower band) and its aggregates (upper band).

Fig. 14A illustrates that a DNA vaccine of the invention can elicit very high titer anti-SARS-CoV sera in mice. Mice 1A-5A were immunized with DNA encoding the S319-518 fragment that contains the spike protein receptor binding domain (RBD). Mice 1B-5B were immunized with RBD-encoding DNA (the S319-518 fragment) fused to a nucleic acid encoding an Fc fragment. Mice 1C-3C received plasmid only (no S fragment DNA). Anti-sera were collected and tested via ELISA to ascertain the titer of the different isolates. In Fig. 14A, the first number denotes an individual mouse, the letter denotes the

respective immunization group, and the last number denotes the dilution used. Anti-sera were diluted by factors of 50, 250, 1250 and 7250, as shown on the x-axis of the bar graph. These data indicate that immunization with DNA encoding the receptor binding domain of the S protein induces a strong immune response against SARS-CoV.

Fig. 14B illustrates that anti-sera from mice immunized with RBD-encoding DNA can prevent S-mediated cell fusion. Cells (293T) were incubated with anti-sera from mice immunized with DNA encoding a spike protein receptor binding domain polypeptide (S319-518) fragment and then the cell suspension was mixed with cells expressing S protein. Fusion was measured as described in Example 20 (see also, Xiao et al. BBRC 2003). The percentage (where 1=100%) of activity for each fusion reaction is plotted on the y-axis, where the percentage of the fusion without any inhibition was designated as 100%. PC denotes positive control where no serum was added. For mice sera #1 to #2 in each group, serum dilution factors of 10 (designated 0.1), 100 (designated 0.01), and 1000 (designated (0.001) were used. For mice sera #3-#5 in groups A and B, and #3 in the control group, dilution factors of 20 (designated 0.05) and 100 (designated 0.01) were used. These data indicate that immunization with DNA encoding the receptor binding domain of the S protein could prevent SARS-CoV infection.

Fig. 15 illustrates that soluble S glycoprotein fragments inhibit S-mediated cell fusion. 10 ug/ml of various S fragments were incubated with ACE2-expressing cells first for 10 min at room temperature. The ACE2-expressing cells were then mixed with S expressing cells and the fusion assay was carried out as described in the Examples. The Y-axis is the OD₅₉₅ for each sample after the background noise was subtracted. Numbers of each construct represent the starting and ending residues of the respective polypeptide.

Detailed Description of the Invention

SARS represents an important public health concern. Methods to diagnose and treat persons who are infected with SARS-CoV provide the opportunity to either prevent or control further spread of infection by SARS-CoV. These methods are especially important due to the ability of SARS-CoV to infect persons through an airborne route. The present invention provides

nucleic acids that encode segments of the amino acid sequence of the spike protein of SARS-CoV. The present invention also provides polypeptides that correspond in amino acid sequence to segments of the amino acid sequence of the spike protein of SARS-CoV. The invention also provides peptide fragments and conservative variants of the spike protein of SARS-CoV, in addition to coupled proteins and peptidomimetics that have portions which correspond in amino acid sequence to the spike protein.

The spike protein is important because it is present on the outside of intact SARS-CoV. Thus, it presents a target that can be used to inhibit or eliminate an intact virus before the virus has an opportunity to infect a cell.

The nucleic acids and polypeptides of the invention offer advantages over the full length spike protein because the nucleic acids are easy to produce and the polypeptides of the invention are produced in large amounts in soluble form. The polypeptides of the invention offer additional advantages over the native spike protein because they can be made to have increased resistant to degradation when administered to an animal. The polypeptides of the invention can also be formulated to increase their antigenicity to make them more efficient antigens to elicit an immune response when administered to an animal, such as a human.

Accordingly, the invention provides nucleic acids and polypeptide antigens that may be used to formulate vaccines and immune compositions that can be used to immunize and treat persons who are infected with SARS-CoV. In addition, the invention provides antibodies that bind to the spike protein of SARS-CoV which may be used to diagnose, immunize, and treat persons infected with SARS-CoV.

Definitions:

An "adjuvant" is generally defined as a substance that nonspecifically enhances the immune response to an antigen. A variety of adjuvants may be employed with the immunopeptides and immunofragopeptides of this invention. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available as,

for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Mich.); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

An "animal" refers to an organism that can mount an immune response upon antigenic challenge. For example, reptiles, avians, and mammals are able to produce antibodies in response to an antigenic challenge. Antibodies raised in non-human organisms are thought to be useful in diagnostic assays to reduce or eliminate cross-reactivity.

An "aptamer" is a peptide, polypeptide or nucleic acid (RNA or DNA) that binds to a polypeptide or peptide fragment of the invention.

A "carrier protein" refers to a polypeptide that can be coupled with a polypeptide or a peptide fragment of the invention to form a coupled protein. A carrier protein may be coupled to a polypeptide or peptide fragment in order to increase the solubility or the immunogenicity of the polypeptide or peptide fragment. A carrier protein may also be coupled to a polypeptide or peptide fragment to provide a tag which provides for separation or detection of the coupled protein. For example, biotin may be used as a carrier protein that is coupled to a polypeptide or peptide fragment to create a coupled protein which can then be isolated through interaction with avidin, or detected through use of a fluorescently tagged avidin. In another example, a carrier protein that is bound by an antibody can be coupled to a polypeptide or peptide fragment to create a coupled protein that is bound by the antibody which binds to the carrier protein of the coupled protein.

The invention encompasses isolated or substantially purified nucleic acids, peptides, polypeptides or proteins. In the context of the present invention, an "isolated" nucleic acid, DNA or RNA molecule or an "isolated" polypeptide is a nucleic acid, DNA molecule, RNA molecule, or polypeptide that exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid, DNA molecule, RNA molecule or polypeptide may exist in a

purified form or may exist in a non-native environment such as, for example, a transgenic host cell. A "purified" nucleic acid molecule, peptide, polypeptide or protein, or a fragment thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free
5 of chemical precursors or other chemicals when chemically synthesized. In one embodiment, an "isolated" nucleic acid is free of sequences that naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can
10 contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. A protein, peptide or polypeptide that is substantially free of cellular material includes preparations of protein, peptide or polypeptide having less than about 30%, 20%, 10%, or 5%
15 (by dry weight) of contaminating protein. When the protein of the invention, or biologically active portion thereof, is recombinantly produced, preferably culture medium represents less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

The terms polypeptide, peptide and protein are used interchangeably
20 herein.

A peptide or polypeptide "fragment" as used herein refers to a less than full length peptide, polypeptide or protein. For example, a peptide or polypeptide fragment can have is at least about 3, at least about 4, at least about 5, at least about 10, at least about 20, at least about 30, at least about 40 amino
25 acids in length, or single unit lengths thereof. For example, fragment may be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or more amino acids in length. There is no upper limit to the size of a peptide fragment. However, in some embodiments, peptide fragments can be less than about 500 amino acids, less than about 400 amino acids, less than about 300 amino acids or less than about 250 amino acids
30 in length. Preferably the peptide fragment can elicit an immune response when used to inoculate an animal. A peptide fragment may be used to elicit an immune response by inoculating an animal with a peptide fragment in combination with an adjuvant, a peptide fragment that is coupled to an adjuvant, or a peptide fragment that is coupled to arsanilic acid, sulfanilic acid, an acetyl

group, or a picryl group. A peptide fragment can include a non-amide bond, and can be a peptidomimetic.

The term "soluble" as used herein refers to the ability of a polypeptide to be solvated in an aqueous solution. For example, a soluble peptide can be mixed
 5 with an aqueous medium such that at least a detectable portion of the peptide is present in the aqueous medium. The peptide may be detected through use of common techniques, such as absorbance of light, fluorescence, the ability to bind dyes, the ability to reduce silver ions, and the like.

The term "specifically binds" refers to an antibody that binds to a single
 10 epitope, but which does not bind to more than one epitope. Accordingly, an antibody that specifically binds to a polypeptide will bind to an epitope that present on the polypeptide, but which is not present on other polypeptides.

I. Polypeptides, peptide fragments, coupled proteins, immunopeptides, and
 15 peptidomimetics of the invention

The invention provides a polypeptide which has an amino acid sequence that corresponds to the amino acid sequence of the spike protein from the virus (SARS-CoV) that is etiologically linked to severe acute respiratory syndrome (SARS). A representative amino acid sequence is provided by SEQ ID NO: 1,
 20 whose sequence is provided below for easy reference.

```

    1 MFIFLLFLTL TSGSDLDRCT TFDDVQAPNY TQHTSSMRGV
    41 YYPDEIFRSD TLYLTQDLFL PFYSNVTGFH TINHTFGNPV
    81 IPFKDGIYFA ATEKSNVVRG WVFGSTMNNK SQSVIIINNS
   121 TNVVIRACNF ELCDNPFFAV SKPMGTQTHT MIFDNAFNCT
   161 FEYISDAFSL DVSEKSGNFK HLREFVFKNK DGFLYVYKGY
   201 QPIDVVRDLP SGFNTLKPFI KLPLGINITN FRAILTAFSP
   241 AQDIWGTSAA AYFVGYLKPT TFMLKYDENG TITDAVDCSQ
   281 NPLAELKCSV KSFEIDKGIY QTSNFRVVPS GDVVRFPNIT
   321 NLCPFGEVFN ATKFPSVYAW ERKKISNCVA DYSLVYNSTF
   361 FSTFKCYGVS ATKLNLCFS NVYADSFVVK GDDVRQIAPG
   401 QTGVIADYNY KLPDDFMGCV LAWNTRNIDA TSTGNVNYKY
   441 RYLRHGKLRP FERDISNVPF SPDGKPCTPP ALNCYWPLND
   481 YGFYTTTGIG YQPYRVVLS FELLNAPATV CGPKLSTDLI
   521 KNQCVNFFNFN GLTGTGVLTP SSKRFQPFQQ FGRDVSDFTD
  
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561 SVRDPKTSEI LDISPCAFGG VSVITPGTNA SSEVAVLYQD
 601 VNCTDVSTAI HADQLTPAWR IYSTGNNVFQ TQAGCLIGAE
 641 HVDTSYECDI PIGAGICASY HTVSLLRSTS QKSIVAYTMS
 681 LGADSSIAYS NNTIAIPTNF SISITTEVMP VSMAKTSVDC
 5 721 NMYICGDSTE CANLLLQYGS FCTQLNRALS GIAAEQDRNT
 761 REVFAQVKQM YKTPTLKYFG GFNFSQILPD PLKPTKRSFI
 801 EDLLFNKVTI ADAGFMKQYG ECLGDINARD LICAQKFNGI
 841 TVLPPLLTDI MIAAYTAALV SGTATAGWTF GAGAALQIPF
 881 AMQMAYRFNG IGVTONVLYE NQKQIANQFN KAISQIQESL
 (10 921 TTTSTALGKL QDVVNQNAQA LNTLVKQLSS NFGAISSVLN
 961 DILSRDKVE AEVQIDRLIT GRLQSLQTYV TQQLIRAAEI
 1001 RASANLAATK MSECVLGQSK RVDFCGKGYH LMSFPQAAPH
 1041 GVVFLHVTYV PSQERNFTTA PAICHEGKAY FPREGVFVFN
 1081 GTSWFITQRN FFSPQIITTD NTFVSGNCDV VIGIINNTVY
 15 1121 DPLQPELDSF KEELDKYFKN HTSPDVDLGD ISGINASVVN
 1161 IQKEIDRLNE VAKNLNESLI DLQELGKYEY YIKWPWYVWL
 1201 GFIAGLIAIV MVTILLCCMT SCCSCLKGAC SCGSCCKFDE
 1241 DDSEPVLKGV KLHYT

20 The invention also provides peptide fragments which have amino acid
 sequences that correspond to a fragment of the spike protein from the virus
 (SARS-CoV) that is etiologically linked to severe acute respiratory syndrome
 (SARS). Such amino acid sequences include those represented by SEQ ID NOs:
 13, 14, 15, 20-59, and 61-63. The peptide fragments of SEQ ID NO: 1 can also
 25 be three or more amino acids in length, and produce an immune response when
 used to immunize an animal. These peptide fragments are exemplified by those
 that are three amino acids in length, or single amino acid units of greater length,
 such as 4, 5, 6, 7, 8, 9, 10 amino acids in length, and an amino acid sequence that
 lacks one amino acid from the amino acid sequence corresponding to SEQ ID
 30 NO: 1.

The invention also provides coupled proteins having a carrier protein
 coupled to a polypeptide or peptide fragment of the invention. The carrier
 protein may be used to increase the solubility of the coupled protein. The carrier
 protein may also be used to increase the immunogenicity of the coupled protein

to increase production of antibodies that bind to the polypeptide or peptide fragment of the invention. The carrier protein may also be used to provide for the separation or detection of a coupled protein. Accordingly, a coupled protein can be detected or isolated by interaction with other components that bind to the carrier protein portion of the coupled protein. For example, a coupled protein having avidin as a carrier protein can be detected or separated with biotin through use of known methods. Numerous carrier proteins may be used to create coupled proteins of the invention. Examples of such carrier proteins include, keyhole limpet hemacyanin, bovine serum albumin, ovalbumin, mouse serum albumin, rabbit serum albumin, and the like. A carrier protein may be coupled to a polypeptide or peptide fragment of the invention by creation of a fusion protein through use of recombinant methods. A carrier protein may also be coupled to a polypeptide or peptide fragment of the invention through use of chemical linking methods, or through use of a chemical linker. Such coupling methods are known in the art and have been described. Harlow et al., *Antibodies: A Laboratory Manual*, page 319 (Cold Spring Harbor Pub. 1988); Taylor, Protein Immobilization, Marcel Dekker, Inc., New York, (1991).

The invention provides immunopeptides having a polypeptide or a peptide fragment of the invention coupled to arsanilic acid, sulfanilic acid, an acetyl group, or a picryl group. Methods to couple such groups to peptides are known and have been reported. Weigle, *J. Exp. Med.*, 116:913-928 (1962); Weigle, *J. Exp. Med.*, 122:1049-1062 (1965); Weigle, *J. Exp. Med.*, 121:289-308 (1965).

The polypeptides and peptide fragments of the invention may be in glycosylated form, or in unglycosylated form. A polypeptide or peptide fragment of the invention may be soluble or insoluble in aqueous solution. The polypeptides and peptide fragments of the invention may be conservative variants. A conservative variant is a polypeptide or peptide fragment derived from a full-length polypeptide, such as that exemplified by SEQ ID NO: 1, by deletion (so-called truncation), addition, or subtraction of one or more amino acids to the N-terminal and/or C-terminal end of the full-length polypeptide; deletion, addition or subtraction of one or more amino acids at one or more sites in the full-length polypeptide. Such variants may result from, for example, genetic polymorphism or from human manipulation. Methods for such

manipulations are generally known in the art. For example, amino acid sequence variants of SEQ ID NO: 1 can be prepared by mutagenesis of DNA encoding the polypeptide. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel, Proc. Natl. Acad. Sci. USA, 82, 488 (1985); Kunkel et al., Methods in Enzymol., 154:367 (1987); U. S. Patent No. 4,873,192; Walker and Gaastra, eds., Techniques in Molecular Biology, MacMillan Publishing Company, New York (1983) and the references cited therein. Guidance as to appropriate amino acid substitutions may be found in the model of Dayhoff et al., Atlas of Protein Sequence and Structure, Natl. Biomed. Res. Found., Washington, C.D. (1978), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, are preferred. For example, substitution of a hydrophobic amino acid for another, or substitution of a hydrophilic amino acid for another. Routine screening assays can be used to determine if a substituted polypeptide or peptide fragment derived from SEQ ID NO: 1 produces an immune response when administered to a mammal. Examples of such screening assays are well known in the art and include enzyme linked immunosorbant assays, radioimmuno assays, chromium release assays, and the like. Such assays have been described. Harlow et al., Antibodies: A Laboratory Manual, page 319 (Cold Spring Harbor Pub. 1988).

The invention provides peptidomimetics of the polypeptides and peptide fragments of the invention. A peptidomimetic describes a peptide analog, such as those commonly used in the pharmaceutical industry as non-peptide drugs, with properties analogous to those of the template peptide. (Fauchere, J., Adv. Drug Res., 15: 29 (1986) and Evans et al., J. Med. Chem., 30:1229 (1987)). Peptidomimetics are structurally similar to polypeptides or peptide fragments having peptide bonds, but have one or more peptide linkages optionally replaced by a linkage such as, --CH₂NH--, --CH₂S--, --CH₂--CH₂--, --CH=CH-- (cis and trans), --COCH₂--, --CH(OH)CH₂--, and --CH₂SO--, by methods known in the art. Advantages of peptide mimetics over natural polypeptide embodiments may include more economical production, greater chemical stability, altered specificity and enhanced pharmacological properties such as half-life, absorption, potency and efficacy.

The polypeptides, peptide fragments, coupled proteins, and peptidomimetics of the invention can be modified for in vivo use by the addition, at the amino-terminus and/or the carboxyl-terminus, of a blocking agent to decrease degradation in vivo. This can be useful in those situations in which the polypeptide termini tend to be degraded by proteases prior to cellular uptake. Such blocking agents can include, without limitation, additional related or unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the polypeptide, peptide fragment, coupled protein, and peptidomimetic to be administered. This can be done either chemically during the synthesis of the polypeptide, peptide fragment, or coupled protein, or by recombinant DNA technology by methods familiar to artisans of average skill. Alternatively, blocking agents such as pyroglutamic acid, or other molecules known in the art, can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Accordingly, the invention provides polypeptides and peptide fragments that are amino-terminally and carboxyl-terminally blocked.

The ability of a polypeptide or peptide fragment of the invention to produce an immune response may be tested through numerous art recognized methods. For example, for their ability to induce antibody production, or to stimulate a cytotoxic T-lymphocyte response.

The polypeptides and peptide fragments of the invention may be used within screening assays to identify or isolate antibodies that bind to the polypeptides or peptide fragments of the invention, or the spike protein from SARS-CoV. For example, the polypeptides or peptide fragments may be used in phage display assays to isolate antibodies that bind to the polypeptides or peptide fragments. In another example, the polypeptides or peptide fragments of the invention may be bound to a solid support to which antibodies are contacted such that antibodies which bind to the polypeptides or peptide fragments become immobilized on the solid support. These antibodies can be later eluted from the solid support. The polypeptides and peptide fragments of the invention may be used to isolate antibodies according to many other methods known in the art.

Expression systems that may be used for small or large scale production of the, coupled proteins, polypeptides or peptide fragments of the invention

include, but are not limited to, cells or microorganisms that are transformed with a recombinant nucleic acid construct that contains a nucleic acid segment of the invention. Examples of recombinant nucleic acid constructs may include bacteriophage DNA, plasmid DNA, cosmid DNA, or viral expression vectors.

5 Examples of cells and microorganisms that may be transformed include bacteria (for example, *E. coli* or *B. subtilis*); yeast (for example, *Saccharomyces* and *Pichia*); insect cell systems (for example, baculovirus); plant cell systems; or mammalian cell systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, W138, and NIH 3T3 cells). Also useful as host cells are primary or
10 secondary cells obtained directly from a mammal that are transfected with a plasmid vector or infected with a viral vector. Examples of suitable expression vectors include, without limitation, plasmids and viral vectors such as herpes viruses, retroviruses, vaccinia viruses, attenuated vaccinia viruses, canary pox viruses, adenoviruses, adeno-associated viruses, lentiviruses and herpes viruses,
15 among others. Synthetic methods may also be used to produce polypeptides and peptide fragments of the invention. Such methods are known and have been reported. Merrifield, *Science*, 85:2149 (1963).

20 II. Nucleic acid segments, expression cassettes, and nucleic acid constructs of the invention

The present invention provides isolated nucleic acid segments that encode the polypeptides, peptide fragments, and coupled proteins of the invention. The nucleic acid segments of the invention also include segments that encode for the same amino acids due to the degeneracy of the genetic code. For
25 example, the amino acid threonine is encoded by ACU, ACC, ACA and ACG and is therefore degenerate. It is intended that the invention includes all variations of the polynucleotide segments that encode for the same amino acids. Such mutations are known in the art (Watson et al, *Molecular Biology of the Gene*, Benjamin Cummings 1987). Mutations also include alteration of a nucleic
30 acid segment to encode for conservative amino acid changes, for example, the substitution of leucine for isoleucine and so forth. Such mutations are also known in the art. Thus, the genes and nucleotide sequences of the invention include both the naturally occurring sequences as well as mutant forms.

The nucleic acid segments of the invention may be contained within a vector. A vector may include, but is not limited to, any plasmid, phagemid, F-factor, virus, cosmid, or phage in double or single stranded linear or circular form which may or may not be self transmissible or mobilizable. The vector can
5 also transform a prokaryotic or eukaryotic host either by integration into the cellular genome or exist extra-chromosomally (e.g. autonomous replicating plasmid with an origin of replication).

Preferably the nucleic acid segment in the vector is under the control of, and operably linked to, an appropriate promoter or other regulatory elements for
10 transcription in vitro or in a host cell, such as a eukaryotic cell, or a microbe, e.g. bacteria. The vector may be a shuttle vector that functions in multiple hosts. The vector may also be a cloning vector that typically contains one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences can be inserted in a determinable fashion. Such insertion can occur without loss
15 of essential biological function of the cloning vector. A cloning vector may also contain a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Examples of marker genes are tetracycline resistance or ampicillin resistance. Many cloning vectors are commercially available (Stratagene, New England Biolabs, Clontech).

20 The nucleic acid segments of the invention may also be inserted into an expression vector. Typically an expression vector contains prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance gene to provide for the amplification and selection of the expression vector in a bacterial host; regulatory elements that control initiation of transcription such as
25 a promoter; and DNA elements that control the processing of transcripts such as introns, or a transcription termination / polyadenylation sequence.

Methods to introduce nucleic acid segment into a vector are available in the art (Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001)). Briefly, a vector
30 into which a nucleic acid segment is to be inserted is treated with one or more restriction enzymes (restriction endonuclease) to produce a linearized vector having a blunt end, a "sticky" end with a 5' or a 3' overhang, or any combination of the above. The vector may also be treated with a restriction enzyme and subsequently treated with another modifying enzyme, such as a polymerase, an

exonuclease, a phosphatase or a kinase, to create a linearized vector that has characteristics useful for ligation of a nucleic acid segment into the vector. The nucleic acid segment that is to be inserted into the vector is treated with one or more restriction enzymes to create a linearized segment having a blunt end, a
5 "sticky" end with a 5' or a 3' overhang, or any combination of the above. The nucleic acid segment may also be treated with a restriction enzyme and subsequently treated with another DNA modifying enzyme. Such DNA modifying enzymes include, but are not limited to, polymerase, exonuclease, phosphatase or a kinase, to create a nucleic acid segment that has characteristics
10 useful for ligation of a nucleic acid segment into the vector.

The treated vector and nucleic acid segment are then ligated together to form a construct containing a nucleic acid segment according to methods available in the art (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001)).
15 Briefly, the treated nucleic acid fragment and the treated vector are combined in the presence of a suitable buffer and ligase. The mixture is then incubated under appropriate conditions to allow the ligase to ligate the nucleic acid fragment into the vector.

The invention also provides an expression cassette which contains a
20 nucleic acid sequence capable of directing expression of a particular nucleic acid segment of the invention, such as SEQ ID NO: 2, either in vitro or in a host cell. Also, a nucleic acid segment of the invention may be inserted into the expression cassette such that an anti-sense message is produced. The expression cassette is an isolatable unit such that the expression cassette may be in linear form and
25 functional for in vitro transcription and translation assays. The materials and procedures to conduct these assays are commercially available from Promega Corp. (Madison, Wisconsin). For example, an in vitro transcript may be produced by placing a nucleic acid sequence under the control of a T7 promoter and then using T7 RNA polymerase to produce an in vitro transcript. This
30 transcript may then be translated in vitro through use of a rabbit reticulocyte lysate. Alternatively, the expression cassette can be incorporated into a vector allowing for replication and amplification of the expression cassette within a host cell or also in vitro transcription and translation of a nucleic acid segment.

Such an expression cassette may contain one or a plurality of restriction sites allowing for placement of the nucleic acid segment under the regulation of a regulatory sequence. The expression cassette can also contain a termination signal operably linked to the nucleic acid segment as well as regulatory
5 sequences required for proper translation of the nucleic acid segment. The expression cassette containing the nucleic acid segment may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one
10 which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Expression of the nucleic acid segment in the expression cassette may be under the control of a constitutive promoter or an inducible promoter which initiates transcription only when the host cell is exposed to some particular external stimulus.

The expression cassette may include in the 5'-3' direction of transcription,
15 a transcriptional and translational initiation region, a nucleic acid segment and a transcriptional and translational termination region functional in vivo and /or in vitro. The termination region may be native with the transcriptional initiation region, may be native with the nucleic acid segment, or may be derived from another source.

20 The regulatory sequence can be a polynucleotide sequence located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influences the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences can include, but are not limited to, enhancers, promoters,
25 repressor binding sites, translation leader sequences, introns, and polyadenylation signal sequences. They may include natural and synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences. While regulatory sequences are not limited to promoters, some useful regulatory sequences include constitutive promoters, inducible
30 promoters, regulated promoters, tissue-specific promoters, viral promoters and synthetic promoters.

A promoter is a nucleotide sequence which controls the expression of the coding sequence by providing the recognition for RNA polymerase and other factors required for proper transcription. A promoter includes a minimal

promoter, consisting only of all basal elements needed for transcription initiation, such as a TATA-box and/or initiator that is a short DNA sequence comprised of a TATA- box and other sequences that serve to specify the site of transcription initiation, to which regulatory elements are added for control of expression. A promoter may be derived entirely from a native gene, or be composed of different elements derived from different promoters found in nature, or even be comprised of synthetic DNA segments. A promoter may contain DNA sequences that are involved in the binding of protein factors which control the effectiveness of transcription initiation in response to physiological or developmental conditions.

The invention also provides a construct containing a vector and an expression cassette. The vector may be selected from, but not limited to, any vector previously described. Into this vector may be inserted an expression cassette through methods known in the art and previously described (Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001)). In one embodiment, the regulatory sequences of the expression cassette may be derived from a source other than the vector into which the expression cassette is inserted. In another embodiment, a construct containing a vector and an expression cassette is formed upon insertion of a nucleic acid segment of the invention into a vector that itself contains regulatory sequences. Thus, an expression cassette is formed upon insertion of the nucleic acid segment into the vector. Vectors containing regulatory sequences are available commercially and methods for their use are known in the art (Clontech, Promega, Stratagene).

III. Immune compositions and vaccines of the invention

The invention provides immune compositions and vaccines that can be used to produce an immune response against the virus that is etiologically linked to severe acute respiratory syndrome when administered to an animal. The immune response may be a humoral immune response or a cellular immune response.

An immune composition of the invention can include an adjuvant and a nucleic acid, polypeptide, peptide fragment, a peptidomimetic, a coupled protein, an immunopeptide of the invention, or any combination thereof. An immune

composition can contain an adjuvant that is not chemically linked to a polypeptide, peptide fragment, a peptidomimetic, a coupled protein, or an immunopeptide of the invention. An immune composition can contain an adjuvant that is chemically linked to a polypeptide, peptide fragment, a peptidomimetic, a coupled protein, or an immunopeptide of the invention. An immune composition of the invention can also include a pharmaceutically acceptable diluent or carrier.

An immune composition may be manufactured conventionally. In particular, a nucleic acid, polypeptide, peptide fragment, peptidomimetic, coupled protein, immunopeptide, or any combination thereof that is contained in the composition may be combined with a pharmaceutically acceptable diluent or carrier. Examples of pharmaceutically acceptable diluent or carriers include water or a saline solution, such as phosphate-buffered saline (PBS). In general, the pharmaceutically acceptable diluent or carrier is selected on the basis of the mode and route of administration and of standard pharmaceutical practices. Pharmaceutically acceptable diluents and carriers as well as all that is necessary for their use in pharmaceutical compositions are described in Remington's Pharmaceutical Sciences, a standard reference text in this field.

Immune compositions may contain adjuvants as disclosed herein and as known in the art. Aluminum compounds may be used as adjuvants. Such aluminum compounds include, aluminum hydroxide, aluminum phosphate, aluminum hydroxyphosphate, and the like. The nucleic acid, polypeptide, peptide fragment, peptidomimetic, coupled protein, immunopeptide, or any combination thereof may be absorbed or precipitated on an aluminum compound according to standard methods. Other adjuvants include polyphosphazene (WO 95/2415), DC-chol (3-beta-[N-(N', N'-dimethylaminomethane) carbamoyl] cholesterol] (U.S. Pat. No. 5,283,185 and WO 96/14831), QS-21 (WO 88/9336) and RIBI from ImmunoChem (Hamilton, Montana). Immunostimulatory oligonucleotides containing unmethylated CpG dinucleotides ("CpG") are known in the art as being adjuvants when administered by both systemic and mucosal routes (WO 96/02555, EP 468520, Davis et al., *J. Immunol.*, 160:870 (1998); McCluskie and Davis, *J. Immunol.*, 161:4463 (1998). CpG when formulated into immune compositions or vaccines, is generally administered in free solution together with free antigen (WO 96/02555; McCluskie and Davis, *J.*

Immunol., 161:4463 (1998)) or covalently conjugated to an antigen (PCT Publication No. WO 98/16247), or formulated with a carrier such as aluminum hydroxide. (Brazolot-Millan et al., Proc.Natl.Acad.Sci., 95:15553 (1998)).

The invention also provides vaccines that include a nucleic acid,
5 polypeptide, a peptide fragment, a peptidomimetic, a coupled protein, an immunopeptide of the invention, a nucleic acid or any combination thereof. Such vaccines can be formulated as described herein or as known in the vaccine arts. For example, a viral vaccine may be created that expresses a polypeptide, a peptide fragment, or a coupled protein of the invention according to methods
10 known in the art. Examples of viral vectors that may be used include, adenoviruses, herpes viruses, vaccinia viruses, canarypox viruses, and the like. Vaccines can also be formulated as a liposome. Such formulations are known to those skilled in the art. Liposomes: A Practical Approach. RRC New Ed, IRL press (1990).

15 The invention also provides nucleic acid based vaccines that express a polypeptide, a peptide fragment, or a coupled protein of the invention. For example, a nucleic acid vaccine can express a polypeptide having SEQ ID NO: 1, 13, 14, 15, 20-59, 61-63 or a fragment of SEQ ID NO: 1. Inoculation of an animal with a nucleic acid construct that encodes a polypeptide, a peptide
20 fragment, or a coupled protein of the invention may lead to a humoral and cell-mediated immune response to the encoded antigen. It is thought that some bone marrow-derived professional antigen presenting cells are transfected by the nucleic acid construct and the encoded antigen is transcribed and translated into an immunogenic polypeptide that elicits specific responses. A feature of nucleic
25 acid vaccines is that they provide for eliciting strong cytotoxic T-lymphocyte (CTL) responses. These responses occur because the nucleic acid-encoded polypeptides are synthesized in the cytosol of transfected cells. Furthermore, nucleic acid constructs that are produced in bacteria are rich in unmethylated CpG nucleotides that are recognized as foreign by macrophages. Thus, they
30 elicit an innate immune response that enhances adaptive immunity. Therefore, nucleic acid vaccines are effective even when administered without adjuvants.

Direct injection of an expression cassette into living host cells transforms a number of the cells and causes them to express the introduced nucleic acid and thereby express a gene product. The transfected cells may display fragments of

the expressed antigens on their cell surfaces together with major histocompatibility class I (MHC I) or class II (MHC II) complexes.

Nucleic acid constructs can be introduced into cells more efficiently by inducing muscle degeneration prior to the injection of the nucleic acid construct into an animal, including a human (Vitadello et. al., Hum. Gene. Ther., 5:11 (1994) ; Danko and Wolff, Vaccine, 12:1499 (1994); Davis et. al., Hum. Gene. Ther., 4:733 (1993)). For example, such a treatment is thought to increase the efficiency of transfer by up to 40 fold. Two of the most commonly used myonecrotic agents are the local anesthetic bupivacaine, and cardiotoxin (Danko and Wolff, Vaccine, 12:1499 (1994); Davis et. al., Hum. Gene. Ther., 4:733 (1993)). A number of other techniques have been employed to transfer nucleic acid constructs to muscle. Such other techniques include retroviral vectors, adenoviral vectors, and liposomes. However, direct injection of naked nucleic acid appears to be the most efficient of these delivery mechanisms at transferring and expressing foreign nucleic acids in cells.

Nucleic acid constructs can be administered in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to a human or other mammalian subject, e.g., physiological saline. A therapeutically effective amount is an amount of the nucleic acid construct that is capable of producing an immune response (e.g., an enhanced T-cell response or antibody production) in a treated animal. As is well known in the medical arts, the dosage for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for administration of a nucleic acid construct is from approximately 10^6 to 10^{12} copies of the nucleic acid construct. This does can be repeatedly administered, as needed.

Numerous routes of administration may be used to administer nucleic acid constructs. Examples of such routes include intramuscular injection, intravenous, intraperitoneal, intradermal, intranasal and subcutaneous injection of nucleic acid constructs have all resulted in immunization against influenza virus hemagglutinin (HA) in chickens (reviewed in Pardoll and Beckerkleg, Immunity 3 (1995), 165-169). Nucleic acid based vaccines can also be

administered through use of a polymeric, biodegradable microparticle or microcapsule delivery vehicle, sized to optimize phagocytosis by phagocytic cells such as macrophages. For example, PLGA (poly-lacto-co-glycolide) microparticles approximately 1-10 μm in diameter can be used. The nucleic acid construct is encapsulated in these microparticles, which are taken up by macrophages and gradually biodegraded within the cell, thereby releasing the nucleic acid construct. Once released, the nucleic acid is expressed within the cell. Another way to achieve uptake of a nucleic acid construct is through use of liposomes. Such liposomes can be prepared by standard methods. The nucleic acid constructs can be incorporated alone into these delivery vehicles or co-incorporated with tissue-specific antibodies. Alternatively, a molecular conjugate can be prepared that is composed of a nucleic acid construct attached to poly-L-lysine by electrostatic or covalent forces. Poly-L-lysine binds to a ligand that can bind to a receptor on target cells. Cristiano et al. (1995), *J. Mol. Med.* 73, 479). Alternatively, lymphoid tissue specific targeting can be achieved by the use of lymphoid tissue-specific transcriptional regulatory elements (TRE) such as a B lymphocyte, T lymphocyte, or dendritic cell specific TRE. Lymphoid tissue specific TRE are known (Thompson et al., *Mol. Cell. Biol.*, 12:1043 (1992); Todd et al., *J. Exp. Med.*, 177:1663 (1993); Penix et al., *J. Exp. Med.*, 178:1483 (1993)).

The invention also provides microbe based vaccines. Generally, these vaccines relate to microbes that have been transformed with a nucleic acid construct that provides for the expression of a polypeptide, a peptide fragment, or a coupled protein of the invention. For example, *Listeria monocytogenes* may be used as a vector to elicit T-cell immunity. This is because it infects antigen-presenting cells and also because infection originates at the mucosa. Lieberman and Frankel, *Vaccine*, 20:2007-10 (2002). According, *Listeria* may be transformed with a nucleic acid construct that provides for the expression of a polypeptide, a peptide fragment, or a coupled protein that elicits an immune response against the spike protein from the coronavirus that causes severe acute respiratory syndrome. Highly attenuated forms of *Listeria* may be constructed according to methods reported in the art. Lieberman and Frankel, *Vaccine*, 20:2007 (2002). *Salmonella* may also be used as a vector to elicit a cytotoxic T

lymphocyte (CTL) response against the coronavirus that causes severe acute respiratory syndrome. Pasetti et al., Infect Immun., 70:4009 (2002).

An immune composition or vaccine may be administered by any conventional route used in the field of vaccines. For example, an immune composition or vaccine can be administered orally or by intravenous infusion, or injected subcutaneously, intramuscularly, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily. The choice of the administration route depends on a number of parameters such as the nature of the active principle; the identity of the polypeptide, peptide fragment, peptidomimetic, coupled protein, immunopeptide, DNA vaccine; or the adjuvant that is combined with the aforementioned molecules.

Administration of an immune composition may take place in a single dose or in a dose repeated once or several times over a certain period. The appropriate dosage varies according to various parameters. Such parameters include the individual treated (adult or child), the immune composition or antigen itself, the mode and frequency of administration, the presence or absence of adjuvant and, if present, the type of adjuvant and the desired effect (e.g. protection or treatment), as will be determined by persons skilled in the art.

IV. Antibodies and aptamers of the invention

The invention provides antibodies that bind to an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 20-59, 60, 61, 62, 63 or a fragment of SEQ ID NO: 1, or conservative variants thereof. Such antibodies are useful for the diagnosis, immunization against, and treatment of severe acute respiratory syndrome (SARS). In some embodiments, the antibody binds to a peptide having SEQ ID NO:58 or 59. Antibodies that bind to the P540 peptide (SEQ ID NO:59) are highly effective, and can detect spike polypeptides even after extensive dilution. For example, a P540 antibody preparation diluted 1:10,000 could still detect spike polypeptides.

Antibodies can be prepared using an intact polypeptide or peptide fragment of interest as the immunizing antigen. The polypeptide or fragment used to immunize an animal can be derived from translated cDNA or chemical synthesis. A polypeptide or peptide fragment can be coupled to a carrier protein, if desired. Such commonly used carrier proteins which are chemically coupled

to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. A coupled protein can be used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

If desired, polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the polypeptide or peptide fragment to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (Coligan, et al., Unit 9, Current Protocols in Immunology, Wiley Interscience, 1991, incorporated by reference).

It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody.

An antibody suitable for binding to a polypeptide or peptide fragment is specific for at least one portion of a region of the polypeptide. For example, one of skill in the art can use a peptide fragment to generate appropriate antibodies of the invention. Antibodies of the invention include polyclonal antibodies, monoclonal antibodies, and fragments of polyclonal and monoclonal antibodies.

The preparation of polyclonal antibodies is well-known to those skilled in the art (Green et al., Production of Polyclonal Antisera, in Immunochemical Protocols (Manson, ed.), pages 1-5 (Humana Press 1992); Coligan et al., Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters, in Current Protocols in Immunology, section 2.4.1 (1992), which are hereby incorporated by reference). For example, a polypeptide or peptide fragment is injected into an animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animal is bled periodically. Polyclonal antibodies specific for the polypeptide or peptide fragment may then be purified from such antisera by, for example, affinity chromatography using the polypeptide or peptide fragment coupled to a suitable solid support.

The preparation of monoclonal antibodies likewise is conventional (Kohler & Milstein, Nature, 256:495 (1975); Coligan et al., sections 2.5.1-2.6.7;

and Harlow et al., *Antibodies: A Laboratory Manual*, page 726 (Cold Spring Harbor Pub. 1988)), which are hereby incorporated by reference. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by
5 removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established
10 techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (Coligan et al., sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes et al., *Purification of Immunoglobulin G (IgG)*, in *Methods in Molecular Biology*, Vol. 10, pages 79-104 (Humana Press 1992)). Methods of in vitro and
15 in vivo multiplication of monoclonal antibodies is well-known to those skilled in the art. Multiplication in vitro may be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally replenished by a mammalian serum such as fetal calf serum or trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate
20 cells, spleen cells, bone marrow macrophages. Production in vitro provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large scale hybridoma cultivation can be carried out by homogenous suspension culture in an air reactor, in a continuous stirrer reactor, or immobilized or entrapped cell culture. Multiplication in vivo may be
25 carried out by injecting cell clones into mammals histocompatible with the parent cells, e.g., osyngeneic mice, to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristine tetramethylpentadecane prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the
30 animal.

Antibodies can also be prepared through use of phage display techniques. In one example, an organism is immunized with an antigen, such as a polypeptide or peptide fragment of the invention. Lymphocytes are isolated from the spleen of the immunized organism. Total RNA is isolated from the

splenocytes and mRNA contained within the total RNA is reverse transcribed into complementary deoxyribonucleic acid (cDNA). The cDNA encoding the variable regions of the light and heavy chains of the immunoglobulin is amplified by polymerase chain reaction (PCR). To generate a single chain
5 fragment variable (scFV) antibody, the light and heavy chain amplification products may be linked by splice overlap extension PCR to generate a complete sequence and ligated into a suitable vector. *E. coli* are then transformed with the vector encoding the scFV, and are infected with helper phage, to produce phage particles that display the antibody on their surface. Alternatively, to generate a
10 complete antigen binding fragment (Fab), the heavy chain amplification product can be fused with a nucleic acid sequence encoding a phage coat protein, and the light chain amplification product can be cloned into a suitable vector. *E. coli* expressing the heavy chain fused to a phage coat protein are transformed with the vector encoding the light chain amplification product. The disulphide
15 linkage between the light and heavy chains are established in the periplasm of *E. coli*. The result of this procedure is to produce an antibody library with up to 10^9 clones. The size of the library can be increased to 10^{18} phage by later addition of the immune responses of additional immunized organisms that may be from the same or different hosts. Antibodies that recognize a specific antigen can be
20 selected through panning. Briefly, an entire antibody library can be exposed to an immobilized antigen against which antibodies are desired. Phage that do not express an antibody that binds to the antigen are washed away. Phage that express the desired antibodies are immobilized on the antigen. These phage are then eluted and again amplified in *E. coli*. This process can be repeated to enrich
25 the population of phage that express antibodies that specifically bind to the antigen. After phage are isolated that express an antibody that binds to an antigen, a vector containing the coding sequences for the antibody can be isolated from the phage particles and the coding sequences can be recloned into a suitable vector to produce an antibody in soluble form. In another example, a
30 human phage library can be used to select for antibodies, such as monoclonal antibodies, that bind to the spike protein from SARS-CoV. Briefly, splenocytes may be isolated from a human that is infected, or not infected, with SARS-CoV and used to create a human phage library according to methods as described above and known in the art. These methods may be used to obtain human

monoclonal antibodies that bind to the spike protein of SARS-CoV. Phage display methods to isolate antigens and antibodies are known in the art and have been described (Gram et al., Proc. Natl. Acad. Sci., 89:3576 (1992); Kay et al., Phage display of peptides and proteins: A laboratory manual. San Diego: Academic Press (1996); Kermani et al., Hybrid, 14:323 (1995); Schmitz et al., Placenta, 21 Suppl. A:S106 (2000); Sanna et al., Proc. Natl. Acad. Sci., 92:6439 (1995)).

An antibody of the invention may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described (Orlandi et al., Proc. Nat'l Acad. Sci. USA, 86:3833 (1989) which is hereby incorporated in its entirety by reference). Techniques for producing humanized monoclonal antibodies are described (Jones et al., Nature, 321:522 (1986); Riechmann et al., Nature, 332:323 (1988); Verhoeyen et al, Science, 239:1534 (1988); Carter et al., Proc. Nat'l Acad. Sci. USA, 89:4285 (1992); Sandhu, Crit. Rev. Biotech., 12:437 (1992); and Singer et al., J. Immunol., 150:2844 (1993), which are hereby incorporated by reference).

In addition, antibodies of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described (Green et al., Nature Genet., 7:13 (1994); Lonberg et al., Nature,

368:856 (1994); and Taylor et al., Int. Immunol., 6:579 (1994), which are hereby incorporated by reference).

Antibody fragments of the invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the
5 fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups
10 resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described (U.S. patents No. 4,036,945; 4,331,647; and 6,342,221, and references contained therein; Porter, Biochem. J., 73:119 (1959); Edelman et al., Methods
15 in Enzymology, Vol. 1, page 422 (Academic Press 1967); and Coligan et al. at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as
20 the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise, an association of V_H and V_L chains. This association may be noncovalent (Inbar et al., Proc. Nat'l Acad. Sci. USA, 69:2659 (1972)). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as
25 glutaraldehyde (Sandhu, Crit. Rev. Biotech., 12:437 (1992)). Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an
30 expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described (Whitlow et al., Methods: A Companion to Methods in Enzymology, Vol. 2, page 97 (1991); Bird et al., Science, 242:423 (1988), Ladner et al., U.S.

patent No. 4,946,778; Pack et al., Bio/Technology, 11:1271 (1993); and Sandhu, Crit. Rev. Biotech., 12:437 (1992)).

Another form of an antibody fragment is a peptide that forms a single complementarity-determining region (CDR). CDR peptides ("minimal
5 recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (Larrick et al., Methods: A Companion to Methods in Enzymology, Vol. 2, page 106 (1991)).

10 An antibody of the invention may be coupled to a toxin. Such antibodies may be used to treat animals, including humans, that are infected with the virus that is etiologically linked to severe acute respiratory syndrome. For example, an antibody that binds to the spike protein of the coronavirus that is etiologically linked to severe acute respiratory syndrome may be coupled to a tetanus toxin
15 and administered to an animal suffering from infection by the aforementioned virus. The toxin-coupled antibody is thought to bind to a portion of a spike protein presented on an infected cell, and then kill the infected cell.

An antibody of the invention may be coupled to a detectable tag. Such antibodies may be used within diagnostic assays to determine if an animal, such
20 as a human, is infected with SARS-CoV. Examples of detectable tags include, fluorescent proteins (i.e., green fluorescent protein, red fluorescent protein, yellow fluorescent protein), fluorescent markers (i.e., fluorescein isothiocyanate, rhodamine, texas red), radiolabels (i.e., ^3H , ^{32}P , ^{125}I), enzymes (i.e., β -galactosidase, horseradish peroxidase, β -glucuronidase, alkaline phosphatase), or
25 an affinity tag (i.e., avidin, biotin, streptavidin). Methods to couple antibodies to a detectable tag are known in the art. Harlow et al., Antibodies: A Laboratory Manual, page 319 (Cold Spring Harbor Pub. 1988).

The invention also provides aptamers to the polypeptides and peptide fragments of the invention. Aptamers of the invention can be peptide or nucleic
30 acid aptamers. Peptide aptamers are peptides that bind to a polypeptide or peptide fragment of the invention with affinities that are often comparable to those for monoclonal antibody-antigen complexes. Similarly, nucleic acid aptamers are nucleic acids that bind to a polypeptide or peptide fragment of the

invention with strong affinities, for example, affinities that are often comparable to those for monoclonal antibody-antigen complexes.

In one example, nucleic acid aptamers can be isolated through use of a library of random oligonucleotide sequences. The library is screened to
5 ascertain which oligonucleotide binds to the S polypeptides and peptide fragments of the invention. The bound oligonucleotides are eluted from the immobilized polypeptides or peptide fragments and are then amplified by PCR. This process may be repeated to select for aptamers having high affinity for the polypeptides and peptide fragments of the invention. The sequence of the
10 nucleic acid coding for the aptamers can then be determined and cloned into a suitable vector to facilitate production and maintenance of the desired aptamer..

Peptide aptamers can be isolated by mRNA display of a library that contains a promoter, a start codon, a nucleic acid sequence that encodes random peptides. In some embodiments, the DNA library also includes a nucleic acid
15 segment that codes for a histidine tag. This library is transcribed using a suitable polymerase, such as T7 RNA polymerase, after which a puromycin-containing poly A linker is ligated onto the 3' end of the newly formed mRNAs. When these mRNAs are translated *in vitro*, the nascent peptides form covalent bonds to the puromycin of the linker to form an mRNA-peptide fusion molecule. The
20 mRNA-peptide fusion molecules are then purified through use of Ni-NTA agarose and oligo-dT-cellulose. The mRNA portion of the fusion molecule is then reverse transcribed. The double-stranded DNA/RNA-peptide fusion molecules are then incubated with a polypeptide or peptide fragment of the invention and unbound fusion molecules are washed away. The bound fusion
25 molecules are eluted from the immobilized polypeptides or peptide fragments and are then amplified by PCR. This process may be repeated to select for aptamers having high affinity for the polypeptides and peptide fragments of the invention. The sequence of the nucleic acid coding for the aptamers can then be determined and cloned into a suitable vector. Methods for the preparation of
30 peptide aptamers have been described (Wilson et al., Proc. Natl. Acad. Sci., 98:3750 (2001)). Accordingly, the invention provides aptamers that recognize the polypeptides and peptide fragments of the invention.

V. Pharmaceutical compositions of the invention

The invention provides pharmaceutical compositions containing an antibody that binds to an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 20-59, 60, 61, 62, 63 or a fragment of SEQ ID NO: 1, or a conservative variant thereof, and a pharmaceutically acceptable carrier. In some
5 embodiments, the antibody binds to a peptide having SEQ ID NO:58 or 59. Antibodies that bind to the P540 peptide (SEQ ID NO:59) are highly effective, and can detect spike polypeptides even after extensive dilution. For example, a P540 antibody preparation at dilution 1:10,000 could still detect spike polypeptides.

10 The pharmaceutical compositions of the invention may be prepared in many forms that include tablets, hard or soft gelatin capsules, aqueous solutions, suspensions, and liposomes and other slow-release formulations, such as shaped polymeric gels. An oral dosage form may be formulated such that the antibody is released into the intestine after passing through the stomach. Such
15 formulations are described in U.S. Patent No. 6,306,434 and in the references contained therein.

Oral liquid pharmaceutical compositions may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable
20 vehicle before use. Such liquid pharmaceutical compositions may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

An antibody can be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be
25 presented in unit dosage form in ampules, prefilled syringes, small volume infusion containers or multi-dose containers with an added preservative. The pharmaceutical compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Pharmaceutical
30 compositions suitable for rectal administration can be prepared as unit dose suppositories. Suitable carriers include saline solution and other materials commonly used in the art.

For administration by inhalation, an antibody can be conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient

means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, an antibody may take the form of a dry powder composition, for example, a powder mix of a modulator and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator. For intra-nasal administration, an antibody may be administered via a liquid spray, such as via a plastic bottle atomizer.

Pharmaceutical compositions of the invention may also contain other ingredients such as flavorings, colorings, anti-microbial agents, or preservatives. It will be appreciated that the amount of an antibody required for use in treatment will vary not only with the particular carrier selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient. Ultimately the attendant health care provider may determine proper dosage. In addition, a pharmaceutical composition may be formulated as a single unit dosage form.

VI. Method to immunize, treat, and diagnose an animal against severe acute respiratory syndrome

The invention provides a method to immunize an animal against severe acute respiratory syndrome. The method relates to administering a therapeutically effective amount of an antibody that binds to an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 20-59, 60, 61, 62, 63 or a fragment of SEQ ID NO: 1, or a conservative variant thereof to an animal; administering an effective amount of an immune composition to an animal; administering an effective amount of a viral vaccine to an animal; or administering an effective amount of a nucleic acid vaccine to an animal. The animal may be a mammal, such as a human. Methods to administer vaccines and immune compositions have been described herein and are known in the art.

An animal may also be treated for infection by SARS-CoV through passive immunization according to the invention. For example, antibodies that bind to an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 20-55, 60, 61, 62, 63 or a fragment of SEQ ID NO: 1, or a conservative variant thereof
5 may be administered to an animal, such as a human, that is infected with SARS-CoV. Such administration may be suitable in situations where a patient is immune compromised and is unable to mount an effective immune response against SARS-CoV, or to a vaccine or immune composition.

The invention provides a method to diagnose severe acute respiratory
10 syndrome in an animal that involves contacting a biological sample obtained from the animal, such as tissue samples, blood, mucus, or saliva, with an antibody that binds to an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 20-59, 60, 61, 62, 33 or a fragment of SEQ ID NO: 1, and determining if the antibody binds to the biological sample. Diagnostic assays that utilize
15 antibodies to detect the presence of an antigen in a biological sample are well known in the art. Briefly, an antibody of the invention may be immobilized on a surface. A biological sample can then be contacted with the immobilized antibody such that an antigen contained in the sample is bound by the antibody to form an antibody-antigen complex. The sample may then be optionally
20 washed to remove unbound materials. A second antibody of the invention that is coupled to a detectable tag, such as an enzyme or radiolabel, can then be contacted with the antibody-antigen complex such that the enzyme or radiolabel is immobilized on the surface. The detectable tag can then be detected to determine if an antigen was present in the biological sample. In another
25 example, a biological sample can be immobilized on a surface. An antibody of the invention that is coupled to a detectable tag is then contacted with the immobilized biological sample and any unbound material is washed away. The presence of the detectable tag is then detected to determine whether the biological sample contained an antigen. Examples of such assays are well
30 known in the art and include, enzyme-linked immunosorbant assays, radioimmuno assays, and the like.

Nucleic acid based methods may also be used to diagnose severe acute respiratory syndrome. In one example, polymerase chain reaction (PCR) may be used to diagnose SARS-CoV infection. Briefly, a biological sample, such as a

tissue sample, blood, mucus, or saliva, is obtained from an animal. The nucleic acids within the sample are then extracted using common methods, such as organic extraction. The extracted nucleic acids are then mixed with forward and reverse primers that anneal to nucleic acids that encode SARS proteins,
5 polymerase, nucleotides, and typically a buffer that includes components that allow the polymerase to extend the forward and reverse primers using the SARS nucleic acid as a template. The presence of amplified DNA between the forward and reverse primers is then detected to determine if the sample contained SARS originated nucleic acid. Nucleic acid hybridization techniques, such as Northern
10 and Southern blotting, may also be used to detect the presence of SARS nucleic acids in a biological sample.

VII. Kits

The invention provides a kit which contains packaging material and an
15 antibody that binds to an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 45, 46, or 47, 58, 59, 61, 62, 63 or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The kit may also contain a syringe to allow for injection of the antibody contained within the kit into an animal, such as a human. In another embodiment, the invention provides a kit that may contain
20 packaging material, and an antibody that binds to an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 20-59, 60, 61, 62, 63 or a fragment of SEQ ID NO: 1, or a conservative variant thereof that is formulated for administration to an animal, such as a human. In some embodiments, the antibody binds to an amino acid sequence set forth in SEQ ID NO:59. In other embodiments, the
25 antibody binds to an amino acid sequence as set forth in SEQ ID NO:58. Such a kit may optionally contain a syringe to allow for injection of the antibody contained within the kit into an animal, such as a human.

The invention also provides a kit which contains packaging material and DNA vaccine having a DNA molecule or expression vector encoding a
30 polypeptide with an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 45, 46, or 47, 58, 59, 61, 62, 63 or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The kit may also contain a device for administering the DNA vaccine (e.g. a syringe or gene gun) to allow for administration of the vaccine contained within the kit into an animal, such as a human.

The invention also provides a kit which contains packaging material and vaccine composition that includes a polypeptide with an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 45, 46, or 47, 58, 59, 61, 62, 63 or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The kit may also contain a device for administering the vaccine (e.g. a syringe) to allow for administration of the vaccine contained within the kit into an animal, such as a human.

The invention also provides a kit for detecting SARS-CoV infection, which contains packaging material and a polypeptide with an amino acid sequence as set forth in SEQ ID NO: 1, 13, 14, 15, 45, 46, or 47, 58, 59, 61, 62, 63 or a fragment of SEQ ID NO: 1, or a conservative variant thereof. The polypeptide(s) can be immobilized onto a solid support. Such a kit may be used for detection of antibodies directed against the SARS-CoV in the serum of infected animals or humans. The kit can also contain a means for detecting binding of such antibodies to the S polypeptide(s).

VIII. Amino Acid sequence of a full-length spike (S) protein (amino acids 1-1255) from the Tor2 isolate of the SARS-CoV virus

MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSD
 20 TLYLTQDLFLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRG
 WVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHT
 MIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKG
 YQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAAA
 YFVGYLKPTTFMLKYDENGITITDAVDCSQNPLAELKCSVKSFEIDKGIYQ
 25 TSNFRVVPBGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVAD
 YSVLYNSTFFSTFKCYGVSATKLNLDLCSNVYADSFVVKGDDVRQIAPG
 QTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNVNYKYRYLRHGK
 LRPFERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVV
 VLSFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQP
 30 FQQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLY
 QDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYE
 CDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTN
 FSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRAL
 SGIAAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSE

IEDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLT
 DDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTO
 NVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTL
 VKQLSSNFGAISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLI
 5 RAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVV
 FLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFS
 PQIITTDNTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSP
 DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKW
 PWYVWLGFIAGLIAIVMVTILLCCMTSCCCLKGACSCGSCCKFDEDDSE
 10 PVLKGVKLHYT (SEQ ID NO: 1)

IX. Nucleic Acid sequence of a full-length spike (S) protein (nucleotides 1-3768)

ATGTTTATTTTCTTATTATTTCTTACTCTCACTAGTGGTAGTGACCTTG
 15 ACCGGTGCACCACTTTTGATGATGTTCAAGCTCCTAATTACACTCAAC
 ATACTTCATCTATGAGGGGGGTTTACTATCCTGATGAAATTTTATAGAT
 CAGACACTCTTTATTTAACTCAGGATTTATTTCTTCCATTTTATTCTAA
 TGTTACAGGGTTTCATACTATTAATCATACGTTTGGCAACCCTGTCAT
 ACCTTTTAAGGATGGTATTTATTTTGCTGCCACAGAGAAATCAAATGT
 20 TGTCCGTGGTTGGGTTTTTGGTTCTACCATGAACAACAAGTCACAGTC
 GGTGATTATTATTAACAATTCTACTAATGTTGTTATACGAGCATGTAA
 CTTTGAATTGTGTGACAACCCTTTCTTTGCTGTTTCTAAACCCATGGG
 TACACAGACACATACTATGATATTCGATAATGCATTTAATTGCACTTT
 CGAGTACATATCTGATGCCTTTTCGCTTGATGTTTCAGAAAAGTCAGG
 25 TAATTTTAAACACTTACGAGAGTTTGTGTTTAAAAATAAAGATGGGTT
 TCTCTATGTTTATAAGGGCTATCAACCTATAGATGTAGTTCGTGATCT
 ACCTTCTGGTTTTTAACACTTTGAAACCTATTTTAAAGTTGCCTCTTGGT
 ATTAACATTACAAATTTTAGAGCCATTCTTACAGCCTTTTCACCTGCT
 CAAGACATTTGGGGGCACGTCAGCTGCAGCCTATTTTGTGTTGGCTATTTA
 30 AAGCCAACTACATTTATGCTCAAGTATGATGAAAATGGTACAATCAC
 AGATGCTGTTGATTGTTCTCAAATCCACTTGCTGAACTCAAATGCTC
 TGTTAAGAGCTTTGAGATTGACAAAGGAATTTACCAGACCTCTAATTT
 CAGGGTTGTTCCCTCAGGAGATGTTGTGAGATTCCCTAATATTACAAA
 CTTGTGTCCTTTTGGAGAGGTTTTTAATGCTACTAAATCCCTTCTGTC

TATGCATGGGAGAGAGAAAAAAATTTCTAATTGTGTTGCTGATTACTCT
GTGCTCTACAACCTCAACATTTTTTTCAACCTTTAAGTGCTATGGCGTT
TCTGCCACTAAGTTGAATGATCTTTGCTTCTCCAATGTCTATGCAGAT
TCTTTTGTAGTCAAGGGAGATGATGTAAGACAAATAGCGCCAGGACA
5 AACTGGTGTTATTGCTGATTATAATTATAAATTGCCAGATGATTTTCAT
GGGTTGTGTCCTTGCTTGGAATACTAGGAACATTGATGCTACTTCAAC
TGGTAATTATAATTATAAATATAGGTATCTTAGACATGGCAAGCTTA
GGCCCTTTGAGAGAGACATATCTAATGTGCCTTTCTCCCCTGATGGCA
AACCTTGCACCCACCTGCTCTTAATTGTTATTGGCCATTAAATGATT
10 ATGGTTTTTACACCACTACTGGCATTGGCTACCAACCTTACAGAGTTG
TAGTACTTTCTTTTGAACCTTTTAAATGCACCGGCCACGGTTTGTGGAC
CAAATTATCCACTGACCTTATTAAGAACCAGTGTGTCAATTTTAATT
TTAATGGACTCACTGGTACTGGTGTGTTAACTCCTTCTTCAAAGAGAT
TTCAACCATTTCACAATTTGGCCGTGATGTTTCTGATTTCACTGATT
15 CCGTTCGAGATCCTAAAACATCTGAAATATTAGACATTTACCTTGCG
CTTTTGGGGGTGTAAGTGTAATTACACCTGGAACAAATGCTTCATCTG
AAGTTGCTGTTCTATATCAAGATGTAACTGCACTGATGTTTCTACAG
CAATTCATGCAGATCAACTCACACCAGCTTGGCGCATATATTCTACTG
GAAACAATGTATTCCAGACTCAAGCAGGCTGTCTTATAGGAGCTGAG
20 CATGTCGACACTTCTTATGAGTGCGACATTCCTATTGGAGCTGGCATT
TGTGCTAGTTACCATACAGTTTCTTTATTACGTAGTACTAGCCAAAAA
TCTATTGTGGCTTATACTATGTCTTTAGGTGCTGATAGTTCAATTGCTT
ACTCTAATAACACCATTGCTATACCTACTAACTTTTCAATTAGCATT
CTACAGAAGTAATGCCTGTTTCTATGGCTAAAACCTCCGTAGATTGTA
25 ATATGTACATCTGCGGAGATTCTACTGAATGTGCTAATTTGCTTCTCC
AATATGGTAGCTTTTGCACACAATAAATCGTGCACTCTCAGGTATTG
CTGCTGAACAGGATCGCAACACACGTGAAGTGTTGCTCAAGTCAAA
CAAATGTACAAAACCCCAACTTTGAAATATTTTGGTGGTTTTAATTTT
TCACAAATATTACCTGACCCTCTAAAGCCAACTAAGAGGTCTTTTATT
30 GAGGACTTGCTCTTTAATAAGGTGACACTCGCTGATGCTGGCTTCATG
AAGCAATATGGCGAATGCCTAGGTGATATTAATGCTAGAGATCTCAT
TTGTGCGCAGAAGTTCAATGGACTTACAGTGTGTCACCTCTGCTCAC
TGATGATATGATTGCTGCCTACACTGCTGCTCTAGTTAGTGGTACTGC
CACTGCTGGATGGACATTTGGTGCTGGCGCTGCTCTTCAAATACCTTT

TGCTATGCAAATGGCATATAGGTTCAATGGCATTGGAGTTACCCAAA
 ATGTTCTCTATGAGAACCAAAAACAAATCGCCAACCAATTTAACAAG
 GCGATTAGTCAAATTCAAGAATCACTTACAACAACATCAACTGCATT
 GGGCAAGCTGCAAGACGTTGTTAACCAGAATGCTCAAGCATTAACA
 5 CACTTGTTAAACAACCTTAGCTCTAATTTTGGTGCAATTTCAAGTGTGC
 TAAATGATATCCTTTCGCGACTTGATAAAGTCGAGGCGGAGGTACAA
 ATTGACAGGTTAATTACAGGCAGACTTCAAAGCCTTCAAACCTATGT
 AACACAACAATAATCAGGGCTGCTGAAATCAGGGCTTCTGCTAATC
 TTGCTGCTACTAAAATGTCTGAGTGTGTTCTTGGACAATCAAAAAGA
 10 GTTGACTIONTTGTGGAAAGGGCTACCACCTTATGTCCTTCCCACAAGCA
 GCCCCGCATGGTGTGTTGTCTTCTACATGTCACGTATGTGCCATCCCAG
 GAGAGGAACCTCACCACAGCGCCAGCAATTTGTCATGAAGGCAAAGC
 ATACTTCCTCGTGAAGGTGTTTTTGTGTTAATGGCACTTCTTGGTTT
 ATTACACAGAGGAACCTTCTTTTCTCCACAAATAATTACTACAGACAAT
 15 ACATTTGTCTCAGGAAATTGTGATGTCGTTATTGGCATCATTAACAAC
 ACAGTTTATGATCCTCTGCAACCTGAGCTCGACTCATTCAAAGAAGA
 GCTGGACAAGTACTTCAAAAATCATAACATCACCAGATGTTGATCTTG
 GCGACATTTCAAGGCATTAACGCTTCTGTCTCAACATTCAAAAAGAA
 ATTGACCGCCTCAATGAGGTCGCTAAAAATTTAAATGAATCACTCAT
 20 TGACCTTCAAGAATTGGGAAAATATGAGCAATATATTAAATGGCCTT
 GGTATGTTTGGCTCGGCTTCATTGCTGGACTAATTGCCATCGTCATGG
 TTACAATCTTGCTTTGTTGCATGACTAGTTGTTGCAGTTGCCTCAAGG
 GTGCATGCTCTTGTGGTTCTTGCTGCAAGTTTGATGAGGATGACTCTG
 AGCCAGTTCTCAAGGGTGTCAAATTACATTACACATAA (SEQ ID NO:
 25 2)

Example 1

Cloning of the spike protein

The nucleic acid sequence encoding the full length spike protein was
 30 obtained through use of overlapping polymerase chain reaction (PCR).
 Overlapping clones containing fragments of the spike protein were obtained
 from the British Columbia Cancer Agency (Vancouver, British Columbia). The
 following primers were used during the PCR reactions to amplify the nucleic
 acid sequence encoding the full-length spike protein of SARS-CoV: Clone 1:

Forward primer: 5'- A GTC GGA TCC GGT AGG CTT ATC ATT AGA G - 3'
 (SEQ ID NO: 3); Reverse primer: 5'- CCA TCA GGG GAG AAA GGC AC-3
 (SEQ ID NO: 4). Clone 2: Forward primer: 5'- GTG CCT TTC TCC CCT GAT
 GG-3' (SEQ ID NO: 5); Reverse primer: 5'- GAA GAG CAG CGC CAG CAC
 5 C-3' (SEQ ID NO: 6). Clone 3: Forward primer: 5'- GGT GCT GGC GCT GCT
 CTT C-3' (SEQ ID NO: 7); Reverse primer: 5'- A CTG TCT AGA GTT CGT
 TTA TGT GTA ATG-3 (SEQ ID NO: 8).

The nucleic acid segment that resulted from overlapping PCR between
 the nucleic acid segments generated with the above pairs of primers contain
 10 amino acid residues from number 1 to number 1255 of the spike protein of the
 virus (SARS-CoV) that is etiologically linked to severe acute respiratory
 syndrome. The underlined primer sequences represent restriction enzyme
 cutting sites for BamHI and XbaI that were used to clone the amplified fragment
 into pCDNA3(+) (Invitrogen, Carlsbad, California).

15 The full length spike protein gene has been cloned as shown in Fig. 1.
 Fig. 1 shows a gel for the nucleic acid segment encoding the full length spike
 protein inserted into the pCDNA3.1(+) vector that has been digested with the
 restriction enzymes (Lane 2: BamHI and XbaI; Lane 3: HindIII).

20

Example 2

Generation of amino-terminal (S1) and carboxyl-terminal (S2) fragments of the full length spike protein

Computer analysis identified a potential functional separation site
 between the amino-terminus (S1) and the carboxyl-terminus (S2) of the spike
 25 protein. The separation site between S1 and S2 is between positions between
 758 and 761 (⁷⁵⁸RNTR⁷⁶¹) relative to SEQ ID NO: 1. PCR was used to create
 nucleic acids that code for the amino-terminal fragment (S1), and the carboxyl-
 terminal fragment (S2) of the spike protein.

The following primers, S1 forward primer: 5'-AGTC GGA TCC GAC
 30 CGG TGC ACC ACT TTT G-3' (SEQ ID NO: 9), and the reverse primer, S1
 Reverse primer: 5'-AGTC GGG CCC CTG TTC AGC AGC AAT ACC-3'
 (SEQ ID NO: 10), were used to prepare a nucleic acid segment coding for amino
 acid residues 17-757 of the spike protein. Two restriction sites, BamHI and
 ApaI, underlined in the two primers were used to clone the nucleic acid segment

coding for the amino-terminal fragment of the spike protein (S1) gene into the pSecTag2B plasmid for expression.

The following pair of primers, S2 Forward: 5'-ACTG GGATCC GAA GTG TTC GCT CAA GTC-3' (SEQ ID NO: 11), and S2 Reverse: 5'-ACTG
 5 TCTAGA TTG CTC ATA TTT TCC C-3' (SEQ ID NO: 12), were used within a PCR reaction to prepare a nucleic acid segment coding for amino acid residues 762-1189 of the spike protein. Two restriction sites, BamHI and XbaI, underlined in the two primers were used to clone the nucleic acid segment coding for the carboxyl-terminal fragment of the spike protein (S2) gene into
 10 pCDNA3.1(+) plasmid for expression.

To create a fragment containing residues 272-537, the following pair of primers was used for PCR amplification: primer 5' GATCGGATCCGGTACAATCACAG 3' (SEQ ID NO:64) and primer 5' GATCGGGCCCGACACACTGGTTC 3' (SEQ ID NO:65). The amplified
 15 fragment was digested with *Bam*HI and *Apa*I and ligated into pSecTag2B digested with the same restriction enzymes. A schematic diagram of the position of many of the soluble spike protein fragments within the full-length spike protein is provided in Fig. 1B.

In some cases, nucleic acids encoding the S fragments and full-length S
 20 polypeptides had their native leader sequence (spike protein amino acids 1-16, MFIFLLFLTLTSGSDL (SEQ ID NO:60)) replaced with a mouse k chain leader sequence (METDTLLLWVLLLWVPGSTGD) (SEQ ID NO: 16) to permit secretion, as described below.

25 Example 3

Generation of the whole soluble spike protein (sS) lacking the cytoplasmic tail and the transmembrane domain

The following pair of primers were used to generate a nucleic acid segment encoding a fragment of the spike protein (sS) lacking the cytoplasmic
 30 tail having amino acids 17-1189 of SEQ ID NO: 1: S1 Forward: 5'- AGTC GGATCC GAC CGG TGC ACC ACT TTT G-3' (SEQ ID NO: 9), and Reverse: 5' ACTG TCTAGA TTG CTC ATA TTT TCC C-3' (SEQ ID NO: 12).

Example 4**Expression of an amino-terminal and carboxyl-terminal fragment of a spike protein**

Expression will be done by transfecting an expression construct
 5 containing the pSecTag2B or pCDNA3.1(+) plasmid and a nucleic acid insert
 that encodes an amino-terminal (S1), a carboxyl-terminal (S2) fragment, or a
 fragment of the spike protein of SARS-CoV that lacks the cytoplasmic tail and
 the transmembrane domain, into 293 or Vero E6 cells. It is thought that
 10 elimination of the transmembrane domain allows the polypeptides and peptide
 fragments to be soluble in an aqueous solution. Expression efficiency of the
 encoded fragments will then be tested. Once a positive signal is obtained as
 determined with gel analysis, a stably transfected cell line will be generated.
 The full length spike protein, and fragments thereof will be purified according to
 methods that are routinely used with other highly glycosylated proteins. Such as
 15 use of a lentil lectin column for large production. The resulting proteins: soluble
 S1 (sS1), soluble S2 (sS2) and whole soluble S (sS) will have the following
 amino acid sequences. Bold lettering denotes the signal peptide which can be
 cleaved so the excreted protein will not contain it.

20 **Amino acid sequence of a soluble amino-terminal fragment of the spike protein**
(amino acids 17-757)

DRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTDLFLPFYSN
 VTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQS
 VIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHMIFDNAFNCTFEYISD
 25 AFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNT
 LKPIFKLPLGINITNFRAILTAFSQAQDIWGTSAAAYFVGYLKPTTFMLKY
 DENGITDAVDCSQNPLAELKCSVKSFEDKGIYQTSNFRVVP
 SGDVVRF PNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKC
 YGVSATKLNLDLCSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKL
 PD 30 DFMGCVLAWNTRNIDATSTGNYNKYRYLRHGKLRP
 FERDISNVPFSPD GKPCTPPALNCYWPLNDYGFYTTTGIGYQPYR
 VVLSFELLNAPATVCG PKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQ
 PFQQFGRDVSDFDTSV RDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQDVNCTDVSTAIHAD
 QLTPAWRIYSTGNNVFQTQAGCLIGA
 EHVDTSYECDIPIGAGICASYHTV

SLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAMAK
TSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQ (SEQ ID
NO: 13)

5 Amino acid sequence of a soluble carboxyl-terminal fragment of the spike
protein (amino acids 762-1189)

EVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLA
DAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALV
SGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKQIANQ
10 FNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSV
LNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAAT
KMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNF
TTAPAICHEGKAYFPREGVVFVNGTTSWFITQRNFFSPQIITTDNTFVSGNC
DVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
15 NIQKEIDRLNEVAKNLNESLIDLQELGKYEQ (SEQ ID NO: 14)

Amino acid sequence of a soluble spike protein having amino acids 17-757 and
762-1189 of SEQ ID NO: 1 (lacking the signal peptide and the potential
cleavage site)

20 DRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTDLFLPFYSN
VTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVI
IINNSTNVVIRACNFELCDNPFFAVSKPMGTQHTMIFDNAFNCTFEYISD
AFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNT
LKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAAYFVGYLKPTTFMLKY
25 DENGITDAVDCSQNPLAELKCSVKSFEDKGIYQTSNFRVVPSPGDVVRF
PNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKC
YGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLDP
DFMGCVLAWNTRNIDATSTGNVNYKYRYLRHGKLRPFERDISNVPFSPD
GKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVLSFELLNAPATVCG
30 PKLSTDLIKNQCVNFNFENGLTGTGVLTPSSKRFQPFQFGRDVSDFDTSV
RDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQDVNCTDVSTAIHAD
QLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYHTV
SLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAMAK
TSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQDEVFAQVK

QMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQ
 YGECLGDINARDLICAQKFNGLTVLPLLTDDMIAAYTAALVSGTATAG
 WTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQI
 QESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRL
 5 DKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVL
 GQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICH
 EGKAYFPREGVFVFNGTTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINN
 TVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRL
 NEVAKNLNESLIDLQELGKYEQ (SEQ ID NO: 15)

10

Example 5

Generation of additional soluble fragments of the spike protein

The nucleic acid sequence encoding a polypeptide containing amino
 acids 17-757 of SEQ ID NO: 1 was obtained through use of polymerase chain
 15 reaction (PCR). The following primers were used during the PCR reactions to
 amplify the nucleic acid sequence: Forward primer: 5' AGCT GGA TCC GAC
 CGG TGC ACC ACT TTT G 3' (SEQ ID NO: 9); and Reverse primer: 5'
 AGCT GGG CCC CTG TTC AGC AGC AAT ACC 3' (SEQ ID NO: 10). The
 resulting PCR product was digested with BamHI and ApaI and, encodes a
 20 polypeptide having an amino acid sequence corresponding to SEQ ID NO: 43.
 The digested PCR product was then ligated to pSecTag2B (Invitrogen, Carlsbad,
 California) that was digested with the same enzymes. The pSecTag2B construct
 containing the PCR product insert encodes a polypeptide having SEQ ID NO: 46
 with the mouse k chain leader sequence (METDTLLLWVLLLWVPGSTGD)
 25 (SEQ ID NO: 16) at the N-terminus for secretion, and a myc epitope
 (EQKLISEEDL) (SEQ ID NO: 17) plus a histidine tag (HHHHHH) (SEQ ID
 NO: 18) at the C-terminus for affinity purification.

The nucleic acid sequence encoding a polypeptide containing amino
 acids 17-276 of SEQ ID NO: 1 was obtained through use of polymerase chain
 30 reaction (PCR). The following primers were used during the PCR reactions to
 amplify the nucleic acid sequence: Forward primer: 5' AGCT GGA TCC GAC
 CGG TGC ACC ACT TTT G 3' (SEQ ID NO: 9); and Reverse primer: 5'
 CTAG CTC GAG CAA CAG CAT CTG TG 3' (SEQ ID NO: 19). The
 resulting PCR product was digested with BamHI and XhoI and, encodes an

amino acid having SEQ ID NO: 44. The digested PCR product was then ligated to pSecTag2B (Invitrogen, Carlsbad, California) that was digested with the same enzymes. The pSecTag2B construct containing the PCR product insert encodes a polypeptide having SEQ ID NO: 47 with the mouse k chain leader sequence
5 (METDTLLLWVLLLWVPGSTGD) (SEQ ID NO: 16) at the N-terminus for secretion, and a myc epitope (EQKLISEEDL) (SEQ ID NO: 17) plus a histidine tag (HHHHHH) (SEQ ID NO: 18) at the C-terminus for affinity purification.

The nucleic acid sequence encoding a polypeptide containing amino acids 17-537 of SEQ ID NO: 1 was obtained by digesting the nucleic acid
10 sequence that encodes SEQ ID NO: 43 (as described above) with BamHI and HincII. The nucleic acid segment produced encodes a polypeptide having SEQ ID NO: 45. This nucleic acid segment was ligated into a pSecTag2B vector that was digested with BamHI and EcoRV. The pSecTag2B construct containing the PCR product insert encodes a polypeptide having SEQ ID NO: 48 with the
15 mouse k chain leader sequence (METDTLLLWVLLLWVPGSTGD) (SEQ ID NO: 16) at the N-terminus for secretion, and a myc epitope (EQKLISEEDL) (SEQ ID NO: 17) plus a histidine tag (HHHHHH) (SEQ ID NO: 18) at the C-terminus for affinity purification.

The expression of these peptide fragments in mammalian cells is
20 illustrated in Fig. 3. This figure shows that the peptide fragments can be secreted into medium in which cells that express the peptide fragments are grown. Fig. 3 also indicates that the peptide fragments are soluble in aqueous medium.

Table 1Examples of additional peptide fragments of the invention

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
20	1-100	MFIFLLFLTLTSGSDL DRCTTFDDVQAP NYTQHTSSMRGVYYPDEIFRSDTLYLTQ DLFLPFYSNVTGFHTINHTFGNPVIPFKD GIYFAATEKSNVVRG
21	101-200	WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQTHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGY
22	201-300	QPIDVVRDLPSGFNTLKPIFKLPLGINITN FRAILTAFSPAQDIWGTSAAYFVGYLK PTTFMLKYDENGITITDAVDCSQNPLAEL KCSVKSFIDKGIY
23	301-400	QTSNFRVVPSPGDVVRFPNITNLCPFGEVF NATKFPSVYAWERKKISNCVADYSVLY NSTFFSTFKCYGVSATKLNDLCFSNVYA DSFVVKGDDVRQIAPG
24	401-500	QTGVIADYNYKLPDDFMGCVLAWNTRN IDATSTGNYNYKYRYLRHGKLRPFERDI SNVPFSPDGKPCTPPALNCYWPLNDYGF YTTTGIGYQPYRVVLS
25	501-600	FELNAPATVCGPKLSTDLIKNCQCVNFN FNGLTGTGVLTPSSKRFQPFQQFGRDVS DFTDSVRDPKTSEILDISPCAFGGVSVITP GTNASSEVAVLYQD
26	601-700	VNCTDVSTAIHADQLTPAWRIYSTGNNV FQTQAGCLIGAETHVDTSYECDIPIGAGIC ASYHTVSLLRSTSQKSIVAYTMSLGADS SIAYSNNTIAIPTNF
27	701-800	SISITTEVMPVSMAKTSVDCNMYICGDST ECANLLLQYGSFCTQLNRALSGIAAEQD RNTREVFAQVKQMYKTPTLKYFGGFNF SQILPDPLKPTKRSFI
28	801-900	EDLLFNKVTLADAGFMKQYGECLGDIN ARDLICAQKFNGLTVLPPLLTDDMIAAY TAALVSGTATAGWTFGAGAALQIPFAM QMAYRFNGIGVTQNVLYE

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
29	901-1000	NQKQIANQFNKAISQIQESLTTTSTALGK LQDVVNQNAQALNTLVKQLSSNFGAISS VLNDILSRLDKVEAEVQIDRLITGRLQSL QTYVTQQLIRAAEI
30	1001-1100	RASANLAATKMSECVLGQSKRVDFCGK GYHLMSEFPQAAPHGVVFLHVTYVPSQE RNFTTAPAICHEGKAYFPREGVVFVNGT SWFITQRNFFSPQIITTD
31	1101-1189	NTFVSGNCDVVIGIINNTVYDPLQPELDS FKEELDKYFKNHTSPDVDLGDISGINASV VNIQKEIDRLNEVAKNLNESLIDLQELGK YEQ
32	1-200	MFIFLLFLTLTSGSD LDRCTTFDDVQAP NYTQHTSSMRGVYYPDEIFRSDTLYLTQ DLFLPFYSNVTGFHTINHTEGPNVIPFKD GIYFAATEKSNVVRGWVFGSTMNNKSQ SVIINNSTNVVIRACNFELCDNPFFAVSK PMGTQTHTMIFDNAFNCTFEYISDAFSLD VSEKSGNFKHLREFVFKNKDGLYVYK GY
33	201-400	QPIDVVRDLPSGFNTLKPIFKLPLGINITN FRAILTAQDIWGTSAAYFVGYLK PTTFMLKYDENGITDAVDCSQNPLAEL KCSVKSFEIDKGIYQTSNFRVVPBGDVVR FPNITNLCPFGEVFNATKFPSVYAWERK KISNCVADYSVLNSTFFSTFKCYGVSA TKLNDLCFSNVYADSFVVKGDDVRQIAP G
34	401-600	QTGVIADYNYKLPDDFMGCVLAWNTRN IDATSTGNYNYKYRYLRHGKLRPFERDI SNVPFSPDGKPCTPPALNCYWPLNDYGF YTTTGIGYQPYRVVLSFELLNAPATVC GPKLSTDLIKNCVNFNFNGLTGTGVLT PSSKRFQPFQQFGRDVSDFTDSVRDPKTS EILDISPCAFGGVSVITPGTNASSEVAVLY QD

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
35	601-800	VNCTDVSTAIHADQLTPAWRIYSTGNNV FQTQAGCLIGA EHVDTSYECDIPIGAGIC ASYHTVSLLRSTSQKSIVAYTMSLGADS SIAYSNNTIAIPTNFSISITTEVMPVSMK TSVDCNMYICGDSTECANLLLQYGSFCT QLNRALSGIAAEQDRNTREVFAQVKQM YKTPTLKYFGGFNFSQILPDPLKPTKRSEI
36	801-1000	EDLLFNKVTLADAGFMKQYGECLGDIN ARDLICAQKFNGLT VLPPLTDDMIAAY TAALVSGTATAGWTFGAGAALQIPFAM QMAYRFNGIGVTQNVLYENQKQIANQF NKAISQIQESLTTTSTALGKLQDVVNQN AQALNTLVKQLSSNFGAISSVLNDILSRL DKVEAEVQIDRLITGRLQSLQTYVTQQLI RAAEI
37	1001-1189	RASANLAATKMSECVLGQSKRVDFCGK GYHLMSEFPQAAPHGVVFLHVTYVPSQE RNFTTAPAICHEGKAYFPREGVVFVNGT SWFITQRNFFSPQIITDNTFVSGNCDVVI GIINNTVYDPLQPELDSFKEELDKYFKNH TSPDVDLGDISGINASVVNIQKEIDRLNE VAKNLNESLIDLQELGKYEQ
38	1-400	MFIFLLFLTLTSGSD LDRCCTTFDDVQAP NYTQHTSSMRGVYYPDEIFRSDTLYL TQ DLFLPFYSNVTGFHTINHTFGNPVIPFKD GIYFAATEKSNVVRGWVFGSTMNNKSQ SVIIINNSTNVVIRACNFELCDNPFFAVSK PMGTQTHMIFDNAFNCTFEYISDAFSLD VSEKSGNFKHLREFVFKNKDGLYVYK GYQPIDVVRDLPSGFNTLKPIFKLPLGINI TNFRAILTAFSPAQDIWGTSAAYFVGY LKPTTFMLKYDENG TITDAVDCSQNPLA ELKCSVKSFEIDKGIYQTSNFRVVPBGDV VRFPNITNLCPFGEVFNATKFPSVYAW RKKISNCVADYSVLYNSTFFSTFKCYGV SATKLNDLCFSNVYADSFVVKGDDVRQI APG

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
39	1-600	MFIFLLFLTLTSGSDL DRCTTFDDVQAP NYTQHTSSMRGVYYPDEIFRSDTLYLTQ DLFLPFYSNVTGFHTINHTFGNPVIPFKD GIYFAATEKSNVVRGWVFGSTMNNKSQ SVIIINNSTNVVIRACNFELCDNPFFAVSK PMGTQHTMIFDNAFNCTFEYISDAFSLD VSEKSGNFKHLREFVFKNKDGFLYVYK GYQPIDVVRDLPSGFNTLKPIFKLPLGINI TNFRAILTAFSPAQDIWGTSAAYFVGY LKPTTFMLKYDENGITITDAVDCSQNPLA ELKCSVKSFEDKGIYQTSNFRVVPBGDV VRFPNITNLCPFGEVFNATKFPSVYawe RKKISNCVADYSVLYNSTFFSTFKCYGV SATKLNDLCFSNVYADSFVVKGDDVRQI APGQTGVIADYNYKLPDDFMGCVLAWN TRNIDATSTGNVNYKYRYLRHGKLRPFE RDISNVPFSPDGKPCTPPALNCYWPLND YGFYTTTGIGYQPYRVVLSFELLNAPA TVCGPKLSTDLIKNCVNFNFNGLTGTG VLTSSKRFQPFQQFGRDVSDFDTSVRDP KTSEILDISPCAFGGVSVITPGTNASSEVA VLYQD

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
40	1-800	MFIFLLFLTLTSGSDL DRCTTFDDVQAP NYTQHTSSMRGVYYPDEIFRSDTLYL TQDLFLPFYSNVTGFHTINHTFGNPV IPFKDGIYFAATEKSNVVRGWVFGSTM NKNKSQSVIIINNSTNVVIRACNFELCD NPFFAVSKPMGTQTHTMIFDNAFNCTF EYISDAFSLDVSEKSGNFKHLREFVFK NKDGFLYVYKGYQPIDVVRDLPSGFNT LKPIFKLPLGINITNFRAILTAFSPAQDI WGTSAAYFVGYLKPTTFMLKYDENG TITDAVDCSQNPLAELKCSVKSFEIDK GIYQTSNFRVVPSGDVVRFPNITNLC PFGFVFNATKFPSVYAWERKKISNCV ADYSVLNSTFFSTFKCYGVSATKLN DLCFSNVYADSFVVKGDDVRQIAPGQ TGVIADYNYKLPDDFMGCVLAWNTRN IDATSTGNYNKYRYLRHGKLRPFERD ISNVPFSPDGKPCTPPALNCYWPLND YGFYTTTGIGYQPYRVVLSFELLNAPA TVCGPKLSTDLIKNCVNFNFNGLTGTG VLTPSSKRFQPFQQFGRDVSDFDTSVR DPKTSEILDISPCAFGGVSVITPGTNAS SEVAVLYQDVNCTDVSTAIHADQLTPAW RIYSTGNNVFQTQAGCLIGAEHVDTSY ECDIPIGAGICASYHTVSLLRSTSQKS IVAYTMSLGADSSIAYSNNTLAIPTNF SISITTEVMPVSMAKTSVDCNMYICGD STECANLLLQYGSFCTQLNRALSGIAAE QDRNTREVFAQVKQMYKTPTLKYFGGF NFSQLPDPLKPTKRSFI

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
41	1-1000	MFIFLLFLTLTSGSDL DRCTTFDDVQAP NYTQHTSSMRGVVYPDEIFRSDTLYLTQ DLFLPFYSNVTGFHTINHTFGNPVIPFKD GIYFAATEKSNVVRGWVFGSTMNNKSQ SVIIINNSTNVVIRACNFELCDNPFFAVSK PMGTQTHTMIFDNAFNCTFEYISDAFSLD VSEKSGNFKHLREFVFKNKDGFLYVYK GYQPIDVVRDLPSGFNTLKPIFKLPLGINI TNFRAILTAFSPAQDIWGTSAAYFVG LKPTTFMLKYDENGTTITDAVDCSQNPLA ELKCSVKSFEIDKGIYQTSNFRVVP SGDVVRFPNITNLCPFGEVFNATKFPSVY AWERKKISNCVADYSVLYNSTFFSTFKCYGV SATKLNLCFSNVYADSFVVKGDDVRQI APGQTGVIADYNYKLPDDFMGCVLAWN TRNIDATSTGNYNKYRYLRHGKLRPFE RDISNVPFSPDGKPCTPPALNCYWPLND YGFYTTTGIGYQPYRVVLSFELLNAPA TVCGPKLSTDLIKNCVNFNFNGLTGTG VLTSSKRFPQFQFGRDVSDFDTSVRDP KTSEILDISPCAFGGVSVITPGTNASSEVA VLYQDVNCTDVSTAIHADQLTPAWRIYS TGNNVFQTQAGCLIGAEHVDTSYECDIPI GAGICASYHTVSLRSTSQKSIVAYTMSL GADSSLAYSNNTIAIPTNFSISITTEVMPVS MAKTSVDCNMYICGDSTECANLLLQYG SFCTQLNRALSGIAAEQDRNTREVFAQV KQMYKTPTLKYFGGFNFSQILPDPLKPT KRSFIEDLLFNKVTLADAGFMKQYGECL GDINARDLICAQKFNGLTVLPPLLTDDMI AAYTAALVSGTATAGWTFGAGAALQIP FAMQMAYRFNGIGVTQNVLYENQKQIA NQFNKAISQIQESLTTTSTALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILS RLDKVEAEVQIDRLITGRLQSLQTYVTQ QLIRAAEI

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
42	1-1189	MFIFLLFLTLTSGSDL DRCTTFDDVQAP NYTQHTSSMRGVYYPDEIFRSDTLYLTO DLFLPFYSNVTGFHTINHTFGNPVIPFKD GIYFAATEKSNVVVRGWVFGSTMNNKSQ SVIIINNSTNVVIRACNFELCDNPFFAVSK PMGTQHTMIFDNAFNCTFEYISDAFSLD VSEKSGNFKHLREFVFKNKDGFYVYK GYQPIDVVRDLPSGFNTLKPIFKLPLGINI TNFRAILTAFSPAQDIWGTSAAYFVG LKPTTFMLKYDENGTTITDAVDCSQNPLA ELKCSVKSFEIDKGIYQTSNFRVVP SGDVVRFPNITNLCPFGEVFNATKFPSVY AWERKKISNCVADYSVLYNSTFFSTFKCYGV SATKLNDLCFSNVYADSFVVKGDDVRQI APGQTGVIADYNYKLPDDFMGCVLAWN TRNIDATSTGNYNKYRYLRHGKLRPFE RDISNVPFSPDGKPCTPPALNCYWPLND YGFYTTTGIGYQPYRVVLSFELLNAPA TVCGPKLSTDLIKNCVNFNFNGLTGTG VLTPSSKRFQPFQFGRDVSDFDTSVRDP KTSEILDISPCAFGGVSVITPGTNASSEVA VLYQDVNCTDVSTAIHADQLTPAWRIYS TGNNVFQTQAGCLIGAEHVDTSYECDIPI GAGICASYHTVSLLRSTSQKSIVAYTMSL GADSSIAYSNNTIAIPTNFSISITTEVMPVS MAKTSVDCNMYICGDSTECANLLQYG SFCTQLNRALSGIAAEQDRNTREVFAQV KQMYKTPTLKYFGGFNFSQILPDPLKPT KRSFIEDLLFNKVTLADAGFMKQYGECL GDINARDLICAQKFNGLTVLPPLTDDMI AAYTAALVSGTATAGWTFGAGAALQIP FAMQMAYRFNGIGVTQNVLYENQKQIA NQFNKAISQIQESLTTTSTALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILS RLDKVEAEVQIDRLITGRLQSLQTYVTQ QLIRAAEIRASANLAATKMSECVLGQSK RVDFCGKGYHLMSFPQAAPHGVVFLHV TYVPSQERNFTTAPAICHEGKAYFPREG VFVFNGTSWFITQRNFFSPQITTDNTFVS GNCDDVIGIINNTVYDPLQPELDSFKEEL DKYFKNHTSPDVDLGDISGINASVVNIQ KEIDRLNEVAKNLNESLIDLQELGKYEQ
43	17-100	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYLTOQLFLPFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVVRG

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
44	17-200	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYL TQDLFL PFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGY
45	17-400	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYL TQDLFL PFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGITIT DAVDCSQNPLAELKCSVKSFEDKGIYQ TSNFRVVPBGDVVRFPNITNLCPFGEVFN ATKFPSVYAWERKKISNCVADYSVLVNS TFFSTFKCYGVSATKLNDLCFSNVYADS FVVKGDVVRQIAPG
46	17-600	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYL TQDLFL PFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGITIT DAVDCSQNPLAELKCSVKSFEDKGIYQ TSNFRVVPBGDVVRFPNITNLCPFGEVFN ATKFPSVYAWERKKISNCVADYSVLVNS TFFSTFKCYGVSATKLNDLCFSNVYADS FVVKGDVVRQIAPGQTGVIADYNYKLDP DFMGCVLAWNTRNIDATSTGNVNYKYR YLRHGKLRPFERDISNVPFSPDGKPCPP ALNCYWPLNDYGFYTTTGIGYQPYRVV VLSFELLNAPATVCGPKLSTDLIKNCQCV NFNENGLTGTGVLTSSKRFQPFQFGR DVSDFTDVSRDPKTSEILDISPCAFGGVS VITPGTNASSEVAVLYQD

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
47	17-800	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYLTQDLFLPFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGITIT DAVDCSQNPLAELKCSVKSFEDKGIYQ TSNFRVVPSPGDVVRFPNITNLCPFGEVFN ATKFPSVYAWERKKISNCVADYSVLYNS TFFSTFKCYGVSATKLNLCFSNVYADS FVVKGDDVRQIAPGQTGVIADYNYKLDP DFMGCVLAWNTRNIDATSTGNVNYKYR YLRHGKLRPFERDISNVPFSPDGKPCPTP ALNCYWPLNDYGFYTTTGIGYQPYRVV VLSFELLNAPATVCGPKLSTDLIKNCV NFNFNGLTGTGVLTSSKRFQPFQFGR DVSDFTDSVRDPKTSEILDISPCAFGGVS VITPGTNASSEVAVLYQDVNCTDVSTAI HADQLTPAWRIYSTGNNVFQTQAGCLIG AEHVDTSYECDIPIGAGICASYHTVSLLR STSQKSIVAYTMSLGADSSIAYSNNITIAIP TNFSISITTEVMPVSMAKTSVDCNMYICG DSTECANLLLQYGSFCTQLNRALSGIAA EQDRNTREVFAQVKQMYKTPTLKYFGG FNFSQILPDPLKPTKRSFI

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
48	17-1000	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYLTQDLFLPFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGTTT DAVDCSQNPLAELKCSVKSFEDKGIYQ TSNFRVVPSPGDVVRFPNITNLCPFGEVFN ATKFPSVYAWERKKISNCVADYSVLYNS TFFSTFKCYGVSATKLNDLCFSNVYADS FVVKGDDVRQIAPGQTGVIADYNYKLDP DFMGCVLAWNTRNIDATSTGNYNYKYR YLRHGKLRPFERDISNVPFSPDGKPCPTP ALNCYWPLNDYGfYTTTGIGYQPYRVV VLSFELLNAPATVCGPKLSTDLIKNCV NFNFNGLTGTGVLTPSSKRFQPFQQFGR DVSDFTDSVRDPKTSEILDISPCAFGGVS VITPGTNASSEVAVLYQDVNCTDVSTAI HADQLTPAWRIYSTGNNVFQTQAGCLIG AEHVDTSYECDIPGAGICASYHTVSLLR STSQKSIVAYTMSLGADSSIAYSNNTIAIP TNFSISITTEVMPVSMAKTSVDCNMYICG DSTECANLLLQYGSFCTQLNRALSGIAA EQDRNTREVFAQVKQMYKTPTLKYFGG FNFSQILPDPLKPTKRSFIEDLLFNKVTLA DAGFMKQYGECLGDINARDLICAQKFN GLTVLPPLLTDDMIAAYTAALVSGTATA GWTFGAGAALQIPFAMQMAYRFNGIGV TQNVLYENQKQIANQFNKAISQIQESLTT TSTALGKLQDVVNQNAQALNTLVKQLS SNFGAISSVLNDILSRDKVEAEVQIDRLI TGRLQSLQTYVTQQLIRAAEI

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
49	17-1189	<p> DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYLTDLFLPFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGITIT DAVDCSQNPLAELKCSVKSFEDKGIYQ TSNFRVVPSPGDVVRFPNITNLCPFGEVFN ATKFPSVYAWERKKISNCVADYSVLYNS TFFSTFKCYGVSATKLNLCFSNVYADS FVVKGDVVRQIAPGQTGVIADYNYKLDP DFMGCVLAWNTRNIDATSTGNYNYKYR YLRHGKLRPFERDISNVPFSPDGKPCPTP ALNCYWPLNDYGFYTTTGIGYQPYRVV VLSFELLNAPATVCGPKLSTDLIKNQCV NFNFNGLTGTGVLTPSSKRFQPFQFGR DVSDFTDSVRDPKTSEILDISPCAFGGVS VITPGTNASSEVAVLYQDVNCTDVSTAI HADQLTPAWRIYSTGNNVFQTQAGCLIG AEHVDTSYECDIPIGAGICASYHTVSLLR STSQKSIVAYTMSLGADSSIAYSNNTIAIP TNFSISITTEVMPVSMAKTSVDCNMYICG DSTECANLLLQYGSFCTQLNRALSGIAA EQDRNTREVFAQVKQMYKTPTLKYFGG FNFSQILPDPLKPTKRSFIEDLLFNKVTLA DAGFMKQYGECLGDINARDLICAQKFN GLTVLPPLLTDDMIAAYTAALVSGTATA GWTFGAGAALQIPFAMQMAYRFNGIGV TQNVLYENQKQIANQFNKAISQIQESLTT TSTALGKLQDVVNQNAQALNTLVKQLS SNFGAISSVLNDILSRDKVEAEVQIDRLI TGRLQSLQTYVTQQLIRAAEIRASANLA ATKMSECVLGQSKRVDFCGKGYHLMSF PQAAPHGVVFLHVTYVPSQERNFTTAPA ICHEGKAYFPREGVVFVNGTSWFITQRNF FSPQIITDNTFVSGNCDVVIGIINNTVYD PLQPELDSFKEELDKYFKNHTSPDVDLG DISGINASVVNIQKEIDRLNEVAKNLNES LIDLQELGKYEQ </p>

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
50	17-276	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYLTDQLFLPFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGITIT DAV
51	17-446	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYLTDQLFLPFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGITIT DAVDCSQNPLAELKCSVKSEIDKGIYQ TSNERVVPSGDVVRFPNITNLCPFGEVFN ATKFPSVYAWERKKISNCVADYSVLYNS TFFSTFKCYGVSATKLNDLCFSNVYADS FVVKGDDVRQIAPGQTGVIADYNYKLPD DFMGCVLAWNTRNIDATSTGNYNYKYR YLRHG

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
52	17-537	DRCTTFDDVQAPNYTQHTSSMRGVYYP DEIFRSDTLYLTLQDLFLPFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVVRG WVFGSTMNNKSQSVIIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGITIT DAVDCSQNPLAELKCSVKSFEDKGIYQ TSNFRVVPSPGDVVRFPNITNLCPFGEVFN ATKFPSVYAWERKKISNCVADYSVLYNS TFFSTFKCYGVSATKLNDLCFSNVYADS FVVKGDDVRQIAPGQTGVIADYNYKLPD DFMGCVLAWNTRNIDATSTGNVNYKYR YLRHGKLRPFERDISNVPFSPDGKPCPTP ALNCYWPLNDYGFYTTTGIGYQPYRVV VLSFELLNAPATVCGPKLSTDLIKNCV NFNENGLTGTGV

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
53	17-757 plus an N-terminal mouse K chain leader sequence and a C- terminal myc epitope and a poly histidine tag	METDTLLLWVLLLWVPGSTGDDRCTTF DDVQAPNYTQHTSSMRGVYYPDEIFRSD TLYLTQDLFLPFYSNVTGFHTINHTFGNP VIPFKDGIYFAATEKSNVVIRGWVFGSTM NNKSQSVIIINNSTNVVIRACNFELCDNPF FAVSKPMGTQHTMIFDNAFNCTFEYIS DAFSLDVSEKSGNFKHLREFVFKNKDGF LYVYKGYQPIDVVRDLPSGFNTLKPIFKL PLGINITNFRAILTAFSQAQDIWGTSAAA YFVGYLKPTTFMLKYDENGTTITDAVDCS QNPLAELKCSVKSFEIDKGIYQTSNFRVV PSGDVVRFPNITNLCPFGEVFNATKFPSV YAWERKKISNCVADYSVLYNSTFFSTFK CYGVSATKLNDLCFSNVYADSFVVKGD DVRQIAPGQTGVIADYNYKLPDDFMGC VLAWNTRNIDATSTGNYNKYRYLRHG KLRPFERDISNVFSPDGKPCPPALNCY WPLNDYGFTTTTGIGYQPYRVVLSFEL LNAPATVCGPKLSTDLIKNCVNFNFNG LTGTGVLTPSSKRFQPFQFGRDVSDF DSVRDPKTSEILDISPCAFGGVSVITPGTN ASSEVAVLYQDVNCTDVSTAIHADQLTP AWRIYSTGNNVFQTQAGCLIGAEHVDTS YECDIPIGAGICASYHTVSLLRSTSQKSIV AYTMSLGADSSIAYSNNTIAIPTNFSISITT EVMPPVSMAKTSVDCNMYICGDSTECAN LLLQYGSFCTQLNRALSGIAAEQEQLIS EEDLHHHHHH
54	17-276 plus an N-terminal mouse K chain leader sequence and a C- terminal myc epitope and a poly histidine tag	METDTLLLWVLLLWVPGSTGDDRCTTF DDVQAPNYTQHTSSMRGVYYPDEIFRSD TLYLTQDLFLPFYSNVTGFHTINHTFGNP VIPFKDGIYFAATEKSNVVIRGWVFGSTM NNKSQSVIIINNSTNVVIRACNFELCDNPF FAVSKPMGTQHTMIFDNAFNCTFEYIS DAFSLDVSEKSGNFKHLREFVFKNKDGF LYVYKGYQPIDVVRDLPSGFNTLKPIFKL PLGINITNFRAILTAFSQAQDIWGTSAAA YFVGYLKPTTFMLKYDENGTTITDAVEQK LISEEDLHHHHHH

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
55	17-537 plus an N-terminal mouse K chain leader sequence and a C- terminal myc epitope and a poly histidine tag	METDTLLLVVLLLVVPGSTGDDRCTTF DDVQAPNYTQHTSSMRGVYYPDEIFRSD TLYLTQDLFLPFYSNVTGFHTINHTFGNP VIPFKDGIYFAATEKSNVVRGWVFGSTM NNKSQSIIINNSTNVVIRACNFELCDNPF FAVSKPMGTQTHTMIFDNAFNCTFEYIS DAFSLDVSEKSGNFKHLREFVFKNKDGF LYVYKGYQPIDVVRDLPSGFNTLKPIFKL PLGINITNFRILTAFSPAQDIWGTSAAA YFVGYLKPTTFMLKYDENGITITDAVDCS QNPLAELKCSVKSEIDKGIYQTSNFRVV PSGDVVRFPNITNLCPFGEVFNATKFPSV YAWERKKISNCVADYSVLYNSTFFSTFK CYGVSATKLNDLCFSNVYADSFVVKG DVRQIAPGQTGVIADYNYKLPDDFMGC VLAWNTRNIDATSTGNVNYKYRYLRHG KLRPFERDISNVPFSPDGKPCPPALNCY WPLNDYGFYTTTGIGYQPYRVVLSFEL LNAPATVCGPKLSTDLIKNCVNFNFNG LTGTGV EQKLISEEDLHHHHHH

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
56	17-756 N-terminal without a signal peptide	DRCTTFDDVQAPNYTQHTSSMRGVVYP DEIFRSDTLYLTLQDLFLPFYSNVTGFHTI NHTFGNPVIPFKDGIYFAATEKSNVVRG WVFGSTMNNKSQSVIINNSTNVVIRACN FELCDNPFFAVSKPMGTQHTMIFDNAF NCTFEYISDAFSLDVSEKSGNFKHLREFV FKNKDGFLYVYKGYQPIDVVRDLPSGFN TLKPIFKLPLGINITNFRAILTAFSQAQDIW GTSAAAYFVGYLKPTTFMLKYDENGITIT DAVDCSQNPLAELKCSVKSFEIDKGIYQ TSNFRVVPSGDVVRFPNITNLCPFGEVFN ATKFPSVYAWEKKISNCVADYSVLVNS TFFSTFKCYGVSATKLNDLCFSNVYADS FVVKGDDVRQIAPGQTGVIADYNYKLDP DFMGCVLAWNTRNIDATSTGNVNYKYR YLRHGKLRPFERDISNVFSPDGKPCPTP ALNCYWPLNDYGFYTTTGIGYQPYRVV VLSFELLNAPATVCGPKLSTDLIKNCV NENFNGLTGTGVLTPSSKRFQPFQFGR DVSDFTDVSRDPKTSEILDISPCAFGGVS VITPGTNASSEVAVLYQDVNCTDVSTAI HADQLTPAWRIYSTGNNVFQTQAGCLIG AEHVDTSYECDIPIGAGICASYHTVSLLR STSQKSIVAYTMSLGADSSIAYSNNTIAP TNFSISITTEVMPVSMAKTSVDCNMYICG DSTECANLLQYGSFCTQLNRALSGIAA E
57	272-537	ITDAVDCSQNPLAELKCSVKSFEIDKGIY QTSNFRVVPSGDVVRFPNITNLCPFGEVFN ATKFPSVYAWEKKISNCVADYSVLVNS TFFSTFKCYGVSATKLNDLCFSNVYADS FVVKGDDVRQIAPGQTGVIADYNYKLDP DFMGCVLAWNTRNIDATSTGNVNYKYR YLRHGKLRPFERDISNVFSPDGKPCPTP ALNCYWPLNDYGFYTTTGIGYQPYRVV VLSFELLNAPATVCGPKLSTDLIKNCV NENFNGLTGTGV
58	24-39 D24 peptide	DVQAPNYTQH TSSMRGC
59	540-555 P540 peptide	PSSKRFQPFQFGRDC

SEQ ID NUMBER	Amino Acid Position Relative to SEQ ID NO: 1	Amino Acid Sequence Amino acid residues in bold (amino acid residues 1-16) identify a signal sequence.
60	1-16 spike signal sequence	MFIFLLFLTLTSGSDL
61	303-537 containing the receptor binding domain	SNFRVVPSGDVVRFPNITNLCPFGEVFNA TKFPSVYAWERKKISNCVADYSVLYNST FFSTFKCYGVSATKLNDLCFSNVYADSF VVKGDDVRQIAPGQTGVIADYNYKLPD DFMGCVLAWNTRNIDATSTGNYNYKYR YLRHGKLRPFERDISNVPFSPDGKPCPTP ALNCYWPLNDYGFYTTTGIGYQPYRVV VLSFELLNAPATVCGPKLSTDLIKNCV NFNENGLTGTGV
62	319-517 containing the receptor binding domain	ITNLCPFGEVFNATKFPSVYAWERKKISN CVADYSVLYNSTFFSTFKCYGVSATKLN DLCFSNVYADSFVVKGDDVRQIAPGQT GVIADYNYKLPDDFMGCVLAWNTRNID ATSTGNYNYKYRYLRHGKLRPFERDISN VPFSPDGKPCPTPALNCYWPLNDYGFYT TTGIGYQPYRVVVLSFELLNAPATVCGP KLST
63	319-518 containing the receptor binding domain	ITNLCPFGEVFNATKFPSVYAWERKKISN CVADYSVLYNSTFFSTFKCYGVSATKLN DLCFSNVYADSFVVKGDDVRQIAPGQT GVIADYNYKLPDDFMGCVLAWNTRNID ATSTGNYNYKYRYLRHGKLRPFERDISN VPFSPDGKPCPTPALNCYWPLNDYGFYT TTGIGYQPYRVVVLSFELLNAPATVCGP KLSTD

Example 6

Structure of the Spike Protein

5 To characterize the properties and function of the SARS-CoV S protein, nucleic acids encoding the full-length Tor2 isolate were cloned into expression vectors as described above. The Tor2 isolate is further described in Marra et al. *The genome sequence of the SARS-associated coronavirus*, Science 300:1399–1404 (2003). Clones generated included the full-length S protein (1255
10 residues), the ectodomain Se (residues 17–1189) having just the extracellular domain of the S protein with the putative transmembrane domain and cytoplasmic tail of the spike protein deleted, fragments containing the N-

terminal 276 (SEQ ID NO:50), 537 (SEQ ID NO:52), and 756 (SEQ ID NO: 56) amino acid residues (S276, S537, and S756, respectively) including a putative 16-residue signal sequence or a mouse k chain leader sequence, and an internal fragment (S272–537) containing residues 272–537 (SEQ ID NO:57) (see Fig. 1B).

Amino acid residues 758–761 (RNTR) form part of the following general motif for cleavage by precursor convertases:



where X is any amino acid residue and $n = 0, 2, 4$ or 6 .

The S1 subunit is approximately encompassed within the S756 fragment. This finding is in agreement with the size of the S1 subunit for murine coronaviruses, e.g., strain JHM where S1 is 769 residues, and for the human coronavirus OC43 (778 residues). See Gallagher & Buchmeier, *Coronavirus spike proteins in viral entry and pathogenesis*, Virology 279: 371–374 (2001); Kunkel & Herrler, *Structural and functional analysis of the surface protein of human coronavirus OC43*, Virology 195 417: 195–202 (1993). However, for the human coronavirus 229E, S1 is considered to consist of a shorter 547 residue fragment that corresponds to S537. Bonavia et al., *Identification of a receptor-binding domain of the spike glycoprotein of human coronavirus HCoV-229E*, J. Virol. 77: 2530–2538 (2003).

All S glycoprotein fragments and the full-length S glycoprotein ran on SDS–PAGE gels at positions significantly higher than their estimated molecular weights, indicating that these polypeptides are likely post-translationally modified. The S276 polypeptide had an apparent molecular weight of about 75 kDa, S537 had an apparent molecular weight of about 100–110 kDa, S756 had an apparent molecular weight of about 130–140 kDa, and Se and S had apparent molecular weights of about 200 kDa or higher (Figs. 4 and 6). The bands corresponding to these polypeptide were broad even when observed at low exposure (Fig. 6; some data not shown). These data indicate significant glycosylation as observed for the S glycoprotein and fragments thereof. Based on approximate estimations of molecular weight it appears that the S2 subunit is not as heavily glycosylated as S756 (constituting the S1 subunit). Notably, S276 is heavily glycosylated if one assumes that only glycosylation contributes to the increased molecular mass.

Most of the SARS-CoV S glycoprotein obtained from cell culture supernatants was not cleaved, although weak bands due to smaller proteins were observed on SDS-PAGE gels. One of these weak bands runs at the same position as S756, suggesting the possibility of inefficient cleavage (Figs. 4 and 6). Random digestion by proteases may occur and further studies are needed to determine if the S glycoprotein cleavage is necessary for its function.

Example 7

Expression of peptide fragments in Escherichia coli

A nucleic acid segment encoding a SEQ ID NO:51 peptide fragment containing amino acid residues 17-446 of SEQ ID NO: 1 was cloned into the pRSET vector (Invitrogen, San Diego, CA) to create the plasmid pRSET-S(17-446). *E. coli* BL21DE3 cells were transformed with pRSET-S(17-446) and then induced with IPTG. The results of the induction are shown in Fig. 2.

Example 8

Use of the T7 promoter to drive expression of a cloned peptide fragment of the invention

Human 293 cells or Monkey Vero E6 cells were grown to a density of 1.2×10^6 cells/T25 flask (60 mm dish) in 5 ml of DMEM+10% FBS medium the day prior to transfection. The cells were then transfected, using the Polyfect (Qiagen) transfection kit according to the manufacturer's protocol, with pSecTag2B constructs (6 ug each) containing inserts coding for the various peptide fragments of the spike protein. These constructs were prepared as described above.

After 4 hour of transfection, a VTF7.3 vaccinia virus carrying a T7 polymerase was used to infect the transfected cells at a MOI (multiplicity of infection) of 20 (Fuerst et al., *Proc. Natl. Acad. Sci.*, **93**:11371 (1986)). This procedure provided for the use of the T7 promoter in the pSecTag2B vector instead of the CMV promoter, which is much weaker (Nussbaum et al., *J. Virol.*, **68**:5411 (1994)). After three hours of infection, 1.5 ml of fresh medium was added to the cells and then the cells were transferred to a 31°C incubator. The cells were incubated for an additional 24 hours, after which the culture medium was collected.

No measurable cytopathicity was observed in cells transfected with any of the S nucleic acid constructs (data not shown), indicating that the full-length and soluble fragments of the S glycoprotein may not have significant cytotoxic effects. However, at higher levels of expression such effects are possible and formation of syncytia as described below may lead to cell death.

Example 9

Spike-Specific Antibodies

New Zealand rabbits were immunized with 0.1 mg of various peptides selected by a computer program for their immunogenicity. Serum from the immunized rabbits was tested in ELISA and Western blot for reactivity. Sera from rabbits immunized with two peptides exhibited the highest and specific activity against the spike glycoprotein and were selected for further study. Antibodies denoted D24 and P540 were elicited by the peptides DVQAPNYTQH TSSMRGC (SEQ ID NO:58) and PSSKRFQPFQQFGRDC (SEQ ID NO:59), respectively. Another anti-SARS-CoV S glycoprotein polyclonal antibody IMG-542, which recognizes amino acid 288–303 of the S glycoprotein, was purchased from IMGENEX (San Diego, CA).

Example 10

Immunoprecipitation and Purification of Spike Polypeptides

Soluble spike polypeptides fragments were obtained from the Vero E6 or 293 cell culture medium. However, the full-length spike glycoprotein was detected only in the cell lysate.

Medium from cells transfected with nucleic acids encoding various soluble S fragments was collected and subjected to centrifugation at 1000g for 10 min to remove cellular debris. The cleared medium was incubated with either Ni-NTA agarose beads (Qiagen, Valencia, CA) or an immunoprecipitating antibody plus glycoprotein G-Sepharose beads (Sigma, St. Louis, MO) for 2 h at 4 °C. The beads were then mixed with an equal volume of SDS gel sample buffer, boiled for 3 min, and subjected to gel analysis. For full-length S glycoprotein, cells were lysed first in PBS supplemented with 1% NP-40 and 0.5mM PMSF for 1 h at 4 °C, and centrifuged at 14,000 rpm in a table-top

Eppendorf centrifuge for 20 min. The cleared lysate was either immunoprecipitated first or used directly in Western blotting.

Example 11

Western blotting and slot blots

5 Cells expressing the S glycoprotein were lysed first with a PBS-based NP40 lysis buffer as described above, and the debris was cleared by centrifugation. For soluble S fragments the medium was collected and cleared as described above. For slot blots, the cleared lysate or medium from supernatant
10 was used directly to blot the nitrocellulose membrane following the protocol suggested by the manufacturer (Bio-Rad, Hercules, CA) and the membrane was subjected to antibody detection as in conventional Western blotting. For Western blotting, a monoclonal anti-c-Myc epitope antibody (Invitrogen, Carlsbad, CA) or anti-spike protein rabbit polyclonal antibodies obtained by
15 immunization of rabbits with spike peptides were diluted in TBST buffer. Antibodies were incubated with the membrane for 2 h, washed and then the membrane was incubated with a secondary antibody conjugated with HRP for 1 h, washed four times (each time for 15 min), and then developed using the ECL reagent (Pierce, Rockford, IL).

20

Example 12

Cell-binding assay and ELISA

Medium containing soluble S fragments was collected and cleared by centrifugation. Vero E6 or other cells (5×10^6) were incubated with 0.5 ml of
25 cleared medium containing soluble S fragments and 2 μ g of anti-c-Myc epitope antibody conjugated with HRP at 4 °C for 2 h. Cells were then washed three times with ice-cold PBS and collected by centrifugation. The cell pellets were incubated with ABTS substrate from Roche (Indianapolis, IN) at RT for 10 min, the substrate was cleared by centrifugation, and the optical density at 405 nm
30 was measured. The result of the slot blot analysis is presented in Fig. 4 and discussed in further detail below.

For ELISA, purified ACE2 (R&D, Minneapolis, MN) was adsorbed onto Maxisorp ELISA plates in pH 9.6 buffer at a concentration of 100 ng per well. Medium 154 (150 μ l) containing various soluble S fragments and 0.6 μ g of anti-

c-155 Myc epitope antibodies conjugated with HRP were incubated in each well at 37 °C for 2 h. Wells were washed and 60 µl of ABTS substrate was added to each well. The optical density (OD₄₀₅) was measured 20 min later.

5

Example 13

Fluorescent dye redistribution cell fusion assay

HeLa or 293T cells, transfected with plasmids encoding the S glycoprotein, were loaded with Calcein AM (Molecular Probes), which is converted within the cells to calcein green. The cells were incubated in medium containing 1 µg/ml Calcein AM for 1 h at 37 °C and 5% CO₂, and then washed and re-suspended in fresh medium. Plated target cells, Vero E6, were stained with CMAC (Molecular Probes) by incubation in 1 µg/ml CMAC in medium for 30 min at 37 °C and 5% CO₂. The cells were then washed twice with medium, incubated for 20 min in fresh medium, washed again, and then covered with 0.5 ml medium per well. The S-expressing cells, loaded with calcein, were added to the target cells and incubated for 1, 2, or 4 h at 37 °C and 5% CO₂. Fusion was measured as the ratio between the cells that have double staining and the total number of target cells in contact with an S glycoprotein-expressing cell. Microphotographs were taken using the MetaMorph 4.0 software from Universal Imaging.

20

Example 14

β-Galactosidase reporter gene-based cell-cell fusion assay

293T cells (1.5x10⁶) were plated in T25 flasks. The next day, these cells were separately transfected with pCDNA3-S, pSectag2B-S, pCDNA3-ACE2, and pCDNA3-ACE2-Ecto using the Polyfect transfection kit (Qiagen, Valencia, CA) following the manufacturer's suggested protocol. Four hours after transfection, cells transfected with S constructs were infected with T7 polymerase-expressing vaccinia virus VTF7.3 and cells transfected with ACE-2 constructs were infected with β-gal encoding vaccinia virus (VCB21R). Two hours after infection, cells were incubated with fresh medium and transferred to 31 °C for overnight incubation. The next day S glycoprotein-expressing cells and ACE-2-expressing cells were mixed in a 1:1 ratio and incubated at 37 °C. Three hours later, cells were lysed by adding NP-40 to a final concentration of 0.5%.

25

30

Cell lysate (50 μ l) was mixed with equal volume of CPRG substrate and OD₅₉₅ was measured 1 hr later.

Example 15

Expression of Spike Polypeptides in Mammalian Cells

For certain experiments, all proteins except the full-length S glycoprotein were tagged with a c-Myc epitope and a histidine tag. These proteins were expressed in 293 and Vero E6 cells after transfection with the corresponding plasmids followed by infection with vaccinia virus-expressing T7 polymerase.

The tagged proteins were detected by using an anti-c-Myc monoclonal antibody (Fig. 4). As shown in Fig. 4, the T7 promoter was a highly efficient promoter for expression of the S glycoprotein. In these experiments, the T7 promoter gave rise to higher levels of expression than the CMV promoter, which under most circumstances is a strong promoter (Fig. 4A). As shown in Fig. 4A, the S fragments were soluble and their concentration in the culture supernatants was inversely proportional to their size.

Example 16

Anti-Spike Antibodies

To be able to detect unlabeled proteins, validate the data obtained by the anti-c-Myc antibody, and localize possible antigenic sites rabbit polyclonal antibodies were developed. Two of these antibodies, D24 and P540, were raised against peptides starting at residues 24 and 540, respectively. The D24 and P540 antibody preparations specifically recognized certain soluble fragments (Fig. 4C). As expected, D24 recognized all fragments; P540 recognized S756, Se, and S but not the smaller fragments (Fig. 4C; some data not shown). The D24 antibody preparation was relatively weak. However, the P540 preparation was very sensitive even at dilution 1:10,000 and was used extensively in the experiments described herein.

The P540 antibody preparation was used to detect whether the S glycoprotein was expressed intracellularly, extracellularly or on the cell surface. As shown in Fig. 5, the full-length S glycoprotein was expressed at the cell surface, although at low levels, as measured by flow cytometry.

Example 17

Spike Protein Mediates Cell Fusion

The full-length S glycoprotein mediates fusion at neutral pH with cells expressing receptor molecules. Cell-cell fusion assays were performed to
5 confirm that the full-length recombinant S glycoprotein was functional, and to ascertain whether the S protein requires other viral proteins and/or low pH for its fusion activity.

Expression of the full-length S glycoprotein with both vectors pCDNA3-S and pSectag2B-S, supported fusion with ACE2 expressing cells efficiently, as
10 evidenced by formation of syncytia of various sizes and by β -gal reporter gene-based assay (Fig. 7). Interestingly, the pSectag2B-S construct in which the S glycoprotein leader peptide was replaced by a mouse k chain leader sequence induced faster formation of syncytia. Moreover, the syncytia formed were larger and more numerous than those induced by pCDNA3-S, which encodes the native
15 S glycoprotein (data not shown). The extent of fusion mediated by S expressed from pSectag2B-S was also higher than from pCDNA3-S as measured by a reporter gene-based assay (Fig. 7B). These data indicate that the natural S glycoprotein may not be efficiently transported to the cell surface. These studies also suggest that the β -gal assay described here can serve as a quick and
20 quantitative method to identify inhibitors of SAR-CoV entry into cells, as well as a tool to study SARS-CoV entry mechanism.

Notably, fusion of Vero E6 cells was not detected using the β -gal assay or the syncytium formation assay when the cells were not transfected with plasmids encoding ACE2 and the cells expressed only native concentrations of
25 the receptor. To explore the possibility that this was due to low sensitivity of these two assays, another assay was used. This new assay was based on fluorescent dye redistribution that is able to detect fusion of single cells. Even with this fluorescent-based assay statistically significant differences between cells transfected with plasmids encoding the full-length S glycoprotein and
30 various negative controls were not detected. Some of the negative controls included transfection with plasmids encoding soluble S fragments at different pH (data not shown). Significant cell-cell fusion was only detected when the cells were transfected with plasmids encoding ACE2, suggesting that the higher levels of receptor expression achieved by expression of recombinant ACE2 could be

important for cell-cell fusion. Overall, these results suggest that recombinant S glycoprotein can mediate cell fusion, that fusion can occur at neutral pH, and that its efficiency is dependent on the concentration of the receptor molecules.

Moreover, soluble fragments of the S glycoprotein inhibit S-mediated cell fusion. As shown in Fig. 15, addition of S fragments S272-537 and S17-537, which have the receptor binding domain as described below, inhibit S-mediated cell fusion. In this assay, the S272-537 (SEQ ID NO:57) fragment, exhibited the most inhibition. The S17-276 fragment that does not have the receptor binding domain exhibited little or no inhibition of S-mediated cell fusion. These data indicate that S polypeptide fragments that have the receptor binding domain could inhibit SARS-CoV fusion with animal cells, thereby inhibiting or preventing SARS-CoV infection.

Hence, blocking, modulating or inhibiting the activity of the spike protein receptor binding domain, with an anti-RBD antibody, S polypeptide, S peptide or aptamer may be an effective preventive or treatment for SARS-CoV infection.

Example 18

Identification of Spike Protein Receptor-Binding Domain

This Example illustrates that the Spike protein receptor-binding domain is localized within residues 272 to 537 (SEQ ID NO:57), and likely within residues 303-537 (SEQ ID NO:61). Later experiments have shown that a fragment containing residues 319-517 (SEQ ID NO:62) also has receptor binding activity.

An assay based on the binding of various soluble fragments to receptor expressing Vero E6 cells was developed to localize the receptor-binding domain (RBD) of the S glycoprotein. This assay involved measurement of fluorescence associated with binding of antibodies directed against the S polypeptides to Vero E6 cells and was developed prior to the identification of the SARS-CoV receptor. Vero E6 cells that are susceptible to SARS-CoV infection were incubated with full-length S polypeptide and various soluble S fragments. Several cell lines that are not susceptible to SARS-CoV infection were similarly incubated with full-length S polypeptides and soluble fragments thereof.

As shown in Figs. 8A and 8B, all fragments S fragments bound to Vero E6 cells except the smallest one S fragment (S276). No such binding was detected when several cell lines that are not susceptible to SARS-CoV infection were incubated with full-length S polypeptides and soluble fragments thereof.

5 Binding to Vero E6 cells was proportional to the expression levels of the fragments and was approximately inversely proportional to the sizes of the fragments. These findings suggested that the RBD is localized between residues 272 and 537.

To further localize the RBD, an antibody (IMG 542) was used that was
10 generated using a peptide containing residues 288–303. This antibody did not inhibit binding of the S537 fragment to Vero E6 cells although it did bind to the S537 fragment (Fig. 8B; some data not shown), suggesting that the RBD is localized between residues 303 and 537. Because of the relatively large antibody size and the possibility for steric hindrance, it is likely that the RBD is
15 downstream of residue 303. Recently, the RBD of the HCoV-229E was localized to a fragment containing amino acid residues 407–547. Ksiazek et al. *A novel coronavirus associated with severe acute respiratory syndrome*, N. Engl. J. Med. 348: 1953–1966 (2003); Rota et al. *Characterization of a novel coronavirus associated with severe acute respiratory syndrome*, Science 300:
20 1394–1399 (2003). In contrast, the RBD for murine hepatitis virus was mapped to the N-terminal 330 amino acids.

It remains to be seen whether there is structural similarity between the RBD-containing fragments of the SARS-CoV S1 glycoprotein (e.g., S272–537) and the HCoV-229E or hepatitis virus RBD, and whether such similarity is
25 related to the use of the same host for replication. These two viruses use different receptors. The straightforward cell-binding approach described here could also be helpful for identification of other virus receptors.

Recently, workers have reported the identification of ACE2 as a functional receptor for the SARS-CoV. Li et al. *Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus*, Nature 426: 450–54 (2003).
30 The identification of ACE2 as receptor permitted further validation that the results provided above are correct. As shown in Fig. 8C, when purified ACE2 is used in an ELISA to test for binding, the same binding pattern was observed as

for the cell-binding assay. This was true for all of the S fragments tested (Fig. 8C).

The results provided herein not only offer new tools to study entry of the SARS virus into cells, confirm that ACE2 is a receptor for the SARS-CoV S1 glycoprotein and localize the RBD but also facilitate development of novel vaccine immunogens and therapeutics for prevention and treatment of SARS.

Example 19

N-terminal and C-terminal Oligomerization of the S glycoprotein

This Example illustrates that the extreme N-terminal fragment of the S glycoprotein, upstream from the RBD, may play a role in fusion, and the S ectodomain forms trimers that could mediate fusion through six-helix bundle intermediates.

Materials and methods

Antibodies and plasmids. The rabbit anti-S serum used in Western and FACS analyses, P540 was developed by the inventors as described above. See also, Xiao et al. Biochem. Biophys. Res. Comm. 312: 1159-65 (2003). The anti-Myc epitope antibody was purchased from Invitrogen (Carlsbad, CA). The anti-ACE2 goat polyclonal antibody was purchased from R&D system (Minneapolis, MN) and used for detection by Western blotting.

Site directed mutagenesis was used to create the consensus cleavage sites corresponding to that of the HIV-1 envelope glycoprotein (Env) and some coronaviruses within the full length SARS-CoV S glycoprotein gene in pCDNA3. The QuickChange Kit from Stratagene (La Jolla, CA) was employed using the protocol provided by manufacturer. For expression of various N terminal S fragments, the corresponding gene fragments were amplified by PCR and cloned into the pSecTag2 expression vector (Invitrogen, Carlsbad). The plasmid pCDNA3-ACE2-ecto, which expresses the ACE2 soluble ectodomain tagged with C9 peptide was kindly provided by Michael Farzan (Harvard University, Boston MA).

Protein expression and purification. Various N terminal fragments of the S glycoprotein were sub-cloned in pSecTag2 expression vector and transfected into 293T cells followed by infection with VTF7.3 as described in Xiao et al.

Biochem. Biophys. Res. Comm. 312: 1159-65 (2003). The protein expressed and secreted into the medium was purified using the HiTrap Ni⁺⁺-Chelating column (Pharmacia) under native conditions. The purified protein was dialyzed against PBS buffer and stored for further analysis.

5 *S glycoprotein dimerization and its interaction with ACE2 examined by co-immunoprecipitation.* For S fragment dimerization, different S glycoprotein constructs, alone or in combination, were transfected to 293T cells as described in Xiao et al. Biochem. Biophys. Res. Comm. 312: 1159-65 (2003). Medium containing S fragments was subjected to immunoprecipitation with rabbit anti-S
10 polyclonal antiserum P540. For some co-immunoprecipitation experiments, DTT was added to create reducing condition to eliminate inter-molecule interactions through disulfide bonds. Immunoprecipitated S fragments were detected by Western using an anti-Myc epitope monoclonal antibodies. Soluble ACE2-C9 was expressed similarly. ACE2-C9 secreted into the medium was
15 used directly for incubation with various S fragments for 2 hours at 4°C. Afterwards, ACE2 was immunoprecipitated by incubating with 1D4 anti-C9 monoclonal antibody and protein G-Sepharose beads at 4°C for one hour. The beads were washed four times with PBS, suspended in SDS-PAGE sample buffer, boiled for 3 min and subjected to gel separation. The presence of either
20 ACE2 or S in the sample was examined by Western as described in Xiao et al. Biochem. Biophys. Res. Comm. 312: 1159-65 (2003).

Flow cytometry. Cells transfected with full length S glycoprotein or S glycoprotein with different N terminal deletions and infected with VTF7.3 were incubated with the P540 rabbit anti-S polyclonal antibody and goat anti-rabbit
25 antibody conjugated with FITC in PBS containing 1% BSA at 4°C for two hours. Cells were then washed four times in ice cold PBS and analyzed with FacsCalibur (Becton Dickinson, San Jose, California).

Gel filtration analysis of S fragments. After being purified on Ni-chelating column and buffer-exchanged to PBS, S fragment samples were loaded
30 onto a Superose 12 10/300 GL column (Pharmacia, Uppsala, Sweden) that had been pre-equilibrated with PBS. The proteins were eluted with PBS at 0.5 ml/min, and 0.5 ml fractions were collected. The Superose 12 column was calibrated with protein molecular mass standard of 669, 440, 232, 158, 67, 44

and 25 kD. A 10 μ l aliquot was taken from each fraction for Western blot analysis.

Crosslinking. Purified S537 fragment was diluted to a concentration of 0.2 μ g/ml in PBS. BS³ (Pierce, Rockford, IL) was added to the S537 solution to
5 a final concentration of 1 mg/ml and incubated on ice for 1 min. The samples were then mixed with an equal volume of 4X SDS-PAGE loading buffer and analyzed by Western blot.

Cell fusion β -gal reporter gene assay. Cells transfected with pSecTag2B-S or pCDNA3-ACE2 and infected with VTF7.3 and VCB21R
10 respectively were collected by trypsin digestion and washed once with PBS. Cells were then suspended in regular DMEM medium at pH 7.4 and mixed. Cells were lysed after four hours of incubation and β -gal activity was measured using CPRG as the substrate (Roche) as described in Xiao et al. Biochem. Biophys. Res. Comm. 312: 1159-65 (2003).

ELISA. Two ELISA assays were used. In the sandwich ELISA the plate
15 was coated with an anti-His tag antibody, then the S fragment were added and detected with an anti-c-Myc epitope antibody. This assay was used for detection of the S fragments. In the second ELISA assay the C9 tagged receptor ACE2 was coated on the plates through an anti-C9 antibody (ID4) and the S fragments
20 were added and after washing detected with an anti-c-Myc epitope antibody. In all experiments the incubations with the c-Myc epitope antibody were for 2 hours at room temperature. The optical density (OD) was measured and normalized to the highest value.

25 Results

The N-terminal fragment upstream of the RBD of the S glycoprotein forms a dimer. It has been previously shown for another coronavirus (MHV) that soluble S1 (similar to SU) fragments form dimers, that the extreme N-terminal 330 amino acid residue region that contains the receptor binding
30 domain participates in the dimerization, and that only dimers bind the receptor CEACAM. See Lewicki & Gallagher, J. Biol. Chem. 277:19727-34 (2002). However, the inventors and others have localized the SARS-CoV receptor binding domain downstream from the extreme N-terminus. Xiao et al. Biochem.

Biophys. Res. Comm. 312: 1159-65 (2003); Wong et al. J. Biol. Chem. 279: 3197-3201 (2004); Babcock et al. J. Virol. 78: 4552-4560 (2004).

To address the possibility of oligomerization by receptor binding domain-containing fragments and to assess their function in mediating membrane fusion, several S fragments were tested for oligomerization. These S fragments included the extreme N-terminal fragment (residues 17 through 276 denoted as S276, SEQ ID NO:50) that does not bind the receptor ACE2, several S fragments (S756, S537, S272-537) that bind ACE2, as well as a fragment including residues 319 through 517 (denoted as S319-517, SEQ ID NO:62) that retains receptor binding activity. These fragments were selected in part because they fold independently and are secreted in the cell culture supernatant, although the efficiency of their expression varied significantly (Fig. 9A, left) and their concentration was decreased when co-expressed with S756 (Fig. 9A, right).

To find whether any of these fragments oligomerizes with the largest one (S756) that includes the equivalent of the receptor-binding subunit of the envelope glycoproteins (SU in general and S1 for coronaviruses) the polypeptide fragments were coexpressed, and then the mixtures in the cell culture supernatants were immunoprecipitated with the antibody P540. As described in previous Examples, this rabbit polyclonal antibody preparation was developed against a peptide containing residues 540-555 (SEQ ID NO:59) of the S glycoprotein. The P450 antibody binds the S756 polypeptide but not the other fragments (Fig. 9B, left). All N-terminal fragments except the smallest fragment (S319-517) containing the receptor binding domain were coimmunoprecipitated with S756 by P540 (Fig. 9B, right). To rule out the possibility of nonspecific disulfide bond formation that may lead to coimmunoprecipitation, DTT was included in one of the coimmunoprecipitation experiments. DTT had no effect on either immunoprecipitation or coimmunoprecipitation of secreted S756 (left lanes) or S756+S276 (right lanes) (Fig. 9C, left panel).

To find the size of the oligomers, one of the fragments (S537) was cross-linked with BS³. The right panel of Fig. 9C shows the appearance of a new band with a molecular weight corresponding to a dimer but not of higher order oligomers. To exclude the possibility of artifacts due to cross-linking and further to confirm the formation of dimers, the S537 fragment was also analyzed by gel filtration. Two gel filtration elution peaks were observed: one due to species of

molecular weight of about 230 kDa and the other one of about 110 kDa (Fig. 10A, upper panel) corresponding to a dimer-sized oligomer and a monomer, respectively. In contrast, the smallest fragment containing the receptor binding domain (S319-517) was eluted only as a monomer at about 35 kDa molecular weight (Fig. 2A, lower panel). Overall, these results suggest that soluble SU is a dimer and that the dimerization domain is within the extreme N-terminal region upstream from residue 317 and the receptor binding domain.

The dimeric N terminal region is required for S mediated cell-cell fusion.

Because the putative dimerization domain is upstream from the receptor binding domain within S1 and the fusion machinery is in S2, one might hypothesize that dimerization may not be required for mediation of fusion. To test this hypothesis, two deletion mutants of the full-length S glycoprotein were generated. The N-terminal 103 residues were deleted from one fragments and the N-terminal 311 residues were deleted from another (Fig. 9A), thereby eliminating the presumed dimerization domain. Both mutants did not exhibit any fusion activity compared to the wild type full-length S glycoprotein, which did (Fig. 9A). To test whether a differential level of expression could account for the lack of observable fusogenic activity, the surface and overall levels of expression were measured by flow cytometry and Western blotting. The data from both assays suggested that the level of expression of the two deletion mutants is undistinguishable from that of the wild type (Figs. 11B and C). These results suggest that the extreme N-terminus is required for fusion by a mechanism that may or may not involve dimerization.

Dimeric S1 binds ACE2 much more efficiently than monomeric fragments containing the Receptor Binding Domain. Previous work with another coronavirus (MHV) suggested that only dimeric S1 binds its receptor CEACAM. Lewicki & Gallagher, J. Biol. Chem. 277:19727-734 (2002). Experiments were conducted on SARS-CoV fragments to understand how the dimeric state of the S1 may affect fusion. In particular, binding of S1 fragments in monovalent and bivalent form to ACE2 was observed by using the anti-c-Myc epitope antibody for conversion of monovalent S1 fragments into bivalent ones. One of these S1 fragments (S319-517, SEQ ID NO:62) did not bind to any measurable degree to surface-immobilized ACE2 unless bound by an anti-c-Myc epitope antibody, which converted it into a bivalent molecule in solution before

and during incubation with the receptor (Fig. 12). In contrast, S537 bound to ACE2 without the antibody although the antibody presence increased its binding (Fig. 12). These results suggest that a dimeric state of S1 could contribute to an increased overall affinity that may enhance fusion efficiency.

5 *The soluble S ectodomain is a trimer.* Viral envelope glycoproteins of class I fusion proteins such as hemagglutinin (HA) of influenza are trimeric through the transmembrane domain. Because the SARS-CoV S glycoprotein was recently found to be class I fusion protein, the S2 subunit may facilitate trimerization of the whole S glycoprotein. However, a dimeric S1 with a
10 trimeric S2 could lead to higher order oligomers whose formation depends on the availability of the dimerization binding site in the native S glycoprotein. To test this possibility the size of the soluble S ectodomains (Se) was approximated by gel filtration, where the transmembrane domain and the cytoplasmic tail were deleted. As shown in Fig. 13, a complex having the approximate size of a trimer
15 (MW 512 kDa) was detected. No higher order oligomers were detected. These results not only suggest that the Se fragment and perhaps the full-length membrane-associated S are trimers in their native unbound state but also indicate that the dimerization site in S1 is not readily available for intertrimer interactions.

20 These results indicate the following: 1) the SU subunit of the SARS-CoV S glycoprotein (S1) forms dimers, 2) the dimerization domain does not overlap and is upstream of the receptor binding domain, 3) deletion of the dimerization domain abolishes fusion, 4) dimeric S1 binds receptor molecules much more efficiently than monovalent fragments containing the receptor
25 binding domain, and 5) the soluble S ectodomain forms trimers under gel filtration conditions.

 It has been previously reported that some SU subunits of class I fusion proteins (that bind receptor molecules) can form dimers including, for example, gp120 of the retrovirus HIV-1 and S1 of the coronavirus MHV. Center et al. J.
30 Virol. 74: 4448-55 (2000); Lewicki et al. J. Biol. Chem. 277: 19727-34 (2002). Until the present work, the role of S1 dimerization for mediation of membrane fusion was unclear. It is now generally accepted that soluble ectodomains such as the gp140 protein of the HIV-1 and SIV envelope glycoproteins (Env) form trimers although dimers and tetramers can be observed. Center et al. Proc. Nat'l

Acad. Sci. U.S.A. 98: 14877-82 (2001). Similarly, it appears that at least a possible fusion intermediate quaternary structure of coronaviruses including the SARS-CoV of S2 is trimeric. Liu et al. Lancet 363: 938-947 (2004); Bosch et al. Proc. Nat'l Acad. Sci. U.S.A. 101: 8455-60 (2004). In contrast, some data
5 indicates that the MHV S2 protein is monomeric after dissociation from S1. Lewicki et al. J. Biol. Chem. 277: 19727-34 (2002). Dimer-to-trimer transitions play a critical role in the mechanism of fusion mediated by class II fusion proteins. Thus it has been proposed that changes in the quaternary structure of some coronaviruses may play a role in the fusion mechanism. *Id.* One should
10 note that both the HIV-1 Env and the MHV S glycoproteins are cleaved and the SU can dissociate from the transmembrane subunit, however, such dissociation may not be important for fusion. In contrast, the SARS-CoV S is not cleaved when expressed in membrane associated or soluble form and cleavage may not be required for fusion. Thus, although the SARS-CoV S glycoprotein is a class I
15 fusion protein, the lack of cleavage is an exception from the rule that the Envs of class I fusion proteins are cleaved presumably to confer a metastable high-energy state that could drive the fusion reaction.

This finding that the SU (S1) domain of the SARS-CoV S glycoprotein can form dimers and also forms trimers with the ectodomain of the
20 transmembrane domain (S2) poses an interesting topological situation. Thus, if two of the monomers within a trimer also form a dimer, then the third monomer would still be free to interact with a "free" monomer from another trimer and form a dimer of the two trimers. In another scenario the orientation of each of the monomers in the trimer may not allow formation of dimers in the trimer but
25 leave "free" binding sites for dimerization with monomers from other trimers. In this case one might expect the formation of a network of trimers. Finally, the three-dimensional structure of the trimer may not allow any interactions of the monomer dimerization sites with other monomers in the same or different trimer. The later possibility is supported by the preliminary data provided herein where
30 higher order oligomers were not detected using the described gel filtration conditions. Under those conditions either intratrimer dimerization occurs but the third monomer conformation does not allow interactions with monomers from other trimers or such interactions are too weak to be detected, or the trimer three-dimensional structure is such that it does not allow dimerization interactions.

Data provided herein demonstrate lack of fusion after deletion of portions of the dimerization domain and indicate that the dimerization region may play a role in fusion although its mechanism may not be through dimerization interactions. In addition, under native conditions where the surface concentration of the S glycoprotein can be very high, as seen in electron micrographs, it is possible that dimerization interactions play a role in stabilizing a “network” of interacting molecules perhaps somewhat similar to networks of proteins that mediate entry of class II fusion proteins. Such networks, if any, could increase the avidity of interaction with receptor molecules and perhaps facilitate the formation of the fusion pore structure by providing a pre-assembled network of Env molecules or even provide energy to drive the fusion reaction in the absence of S cleavage that generates a high-energy metastable state.

Example 20

Sera from Mice Immunized with DNA Encoding RBD Polypeptides **Inhibits S-Mediated Cell Fusion**

This Example illustrates that immunizing mammals with DNA encoding receptor binding domain polypeptides may prevent SARS infection.

Materials and Methods

Mice were divided into three groups: group A of mice # 1 through 5 were immunized with plasmid pSecTag-SRBD that encodes for the S319-518 fragment that includes the receptor binding domain (RBD) of the spike protein; group B of mice #1 to #5 were immunized with the plasmid pEAK-10-RBD-Fc that encodes for a fusion protein of RBD (S319-518) fragment fused to Fc and group C mice #1 to #3 which were immunized with a control plasmid. Five BALB/C mice per group were immunized at day 0, day 14 and day 28. Mice received less than 2 ug DNA per immunization with a gene gun. Sera were collected at day 56. In Fig. 14A-B, the first number denotes an individual mouse, the letter denotes the respective immunization group, and the last number denotes the dilution used.

Cells (293T) were incubated with anti-sera from the immunized mice and then mixed with cells expressing S protein. Fusion was measured as described in previous Examples (see also, Xiao et al. BBRC 2003). PC denotes positive

control where no serum was added. For mice #1 to #2 in each group, serum dilution factors of 10, 100, and 1000 were used. For mice #3-#5 in groups A and B, and #3 in the control group, dilution factors of 20 and 100 were used.

5 Results

The antibody titers for the anti-sera obtained from the mice are shown in Fig. 14A. As shown, mice immunized with DNA encoding the spike protein receptor binding domain (S319-518, groups A and B) had very high titer anti-sera – dilutions up to 1:7250 still reacted strongly to antigen in ELISA assays.

10 As shown in FIG. 14B, anti-sera from mice immunized with DNA encoding the spike protein receptor binding domain inhibited fusion of cells that express the S protein in a dose dependent manner. Thus, anti-sera from mouse 1A and 2A, which were immunized with DNA encoding the S receptor binding domain, substantially eliminated S-protein mediated cell fusion when used at a
15 1:10 dilution. Higher dilutions (1:100 and 1:1000) of this anti-sera were less effective. Similar results were observed on cell fusion inhibited by anti-sera from mouse 3A (1:20 dilution), from mouse 4A (1:20 dilution), and from mouse 5A (1:20 dilution).

20 These data indicate that immunizing mammals with DNA encoding S protein receptor binding domain polypeptides can raise a strong immune response against the spike protein and could prevent SARS infection. As described above, soluble fragments of the S glycoprotein that have the receptor binding domain inhibit S-mediated cell fusion (see Fig. 15).

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All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated
25 by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

The specific methods and compositions described herein are
30 representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art

that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a host cell” includes a plurality (for example, a culture or population) of such host cells, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject

matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, 5 those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

WHAT IS CLAIMED:

1. A polypeptide fragment of SEQ ID NO: 1, or a conservative variant thereof, wherein the polypeptide can produce a humoral or cellular immune response when used to inoculate an animal.
2. A polypeptide having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63, wherein the polypeptide, wherein the polypeptide can produce a humoral or cellular immune response when used to inoculate an animal.
3. A polypeptide having any one of SEQ ID NOs: 13, 14, 15, 25, 34, 46, 51, 52, 56, 57, 58, 59, 61, 62 or 63, wherein the polypeptide, wherein the polypeptide can produce a humoral or cellular immune response when used to inoculate an animal.
4. The polypeptide of claim 1, 2 or 3, wherein the polypeptide is soluble in an aqueous solution.
5. The polypeptide of claim 1, 2 or 3, wherein the animal is a mammal.
6. The polypeptide of claim 5, wherein the mammal is a human.
7. The polypeptide of claim 1, 2 or 3, wherein the polypeptide is amino-terminally or carboxyl-terminally blocked.
8. A coupled protein comprising a carrier protein coupled to a second polypeptide having any one of (a) SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a peptide fragment of SEQ ID NO: 1, or a conservative variant of (a) or (b).
9. The coupled protein of claim 8, wherein the carrier protein is soluble in an aqueous solution.

10. The coupled protein of claim 9, wherein the carrier protein is selected from the group consisting of bovine serum albumin, keyhole limpet hemacyanin, ovalbumin, mouse serum albumin, rabbit serum albumin.
- 5 11. The coupled protein of claim 8, wherein the coupled protein produces a humoral or a cellular immune response when used to inoculate an animal.
12. The coupled protein of claim 11, wherein the animal is a mammal.
- 10 13. The coupled protein of claim 12, wherein the mammal is a human.
14. An immunopeptide comprising a polypeptide having any one of (a) SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or (b) a fragment of SEQ ID NO: 1; coupled to arsanilic acid, sulfanilic acid, an acetyl group, or a picryl
15 group.
15. The immunopeptide of claim 14, wherein the immunopeptide produces a humoral or a cellular immune response when used to inoculate an animal.
- 20 16. The immunopeptide of claim 15, wherein the animal is a mammal.
17. The immunopeptide of claim 16, wherein the mammal is a human.
18. An immune composition comprising an adjuvant and a polypeptide
25 having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
19. The immune composition of claim 18, wherein the adjuvant is selected from the group consisting of aluminum hydroxide, lipid A, killed
30 bacteria, polysaccharide, mineral oil, Freund's incomplete adjuvant, Freund's complete adjuvant, aluminum phosphate, iron, zinc, a calcium salt, acylated tyrosine, an acylated sugar, a cationically derivatized polysaccharide, an anionically derivatized polysaccharide, a

polyphosphazine, a biodegradable microsphere, a monophosphoryl lipid A, and quil A.

20. The immune composition of claim 18, wherein the polypeptide is amino-terminally or carboxyl-terminally blocked.
21. A peptidomimetic of an amino acid sequence having any one of (a) SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a fragment of SEQ ID NO: 1, or a conservative variant of (a) or (b).
22. An immune composition comprising an adjuvant and a peptidomimetic of an amino acid sequence having any one of (a) SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a fragment of SEQ ID NO: 1; or a conservative variant of (a) or (b).
23. The immune composition of claim 22, wherein the adjuvant is selected from the group consisting of aluminum hydroxide, lipid A, killed bacteria, polysaccharide, mineral oil, Freund's incomplete adjuvant, Freund's complete adjuvant, aluminum phosphate, iron, zinc, a calcium salt, acylated tyrosine, an acylated sugar, a cationically derivatized polysaccharide, an anionically derivatized polysaccharide, a polyphosphazine, a biodegradable microsphere, a monophosphoryl lipid A, and quil A.
24. A nucleic acid segment that encodes a polypeptide having any one of (a) SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a peptide that is a fragment of SEQ ID NO: 1, or a conservative variant of (a) or (b).
25. An expression cassette comprising a promoter that is operably linked to a nucleic acid segment that encodes a polypeptide having any one of (a) SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a peptide that is a fragment of SEQ ID NO: 1; or a conservative variant of (a) or (b).

26. The expression cassette according to claim 25, wherein the promoter is a constitutive promoter or a regulated promoter.
27. A nucleic acid construct comprising a vector and a nucleic acid segment
5 that encodes (a) a polypeptide having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a peptide that is a fragment of SEQ ID NO: 1; (c) a conservative variant of (a) or (b); or an expression cassette according to claim 25.
- 10 28. The nucleic acid construct according to claim 27, wherein the vector is selected from the group consisting of a plasmid, a cosmid, a yeast artificial chromosome, a bacterial artificial chromosome, an F-factor, a virus, an expression vector, and a phagemid.
- 15 29. A recombinant virus comprising a viral vector and a nucleic acid segment that encodes (a) a polypeptide having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a peptide that is a fragment of SEQ ID NO: 1; (c) a conservative variant of (a) or (b); or an expression cassette according to claim 25.
- 20 30. The recombinant virus of claim 29, wherein the viral vector is selected from the group consisting of vaccinia virus, canarypox, adenovirus, and herpes virus.
- 25 31. A composition comprising a pharmaceutical carrier and (a) a polypeptide having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a peptide that is a fragment of SEQ ID NO: 1; or (c) a conservative variant of (a) or (b).
- 30 32. The composition of claim 31, wherein the composition is formulated for treatment of SARS-CoV.
33. The composition of claim 31, wherein the composition is formulated for inhibition of SARS-CoV fusion with, or entry into, mammalian cells.

34. A composition comprising a pharmaceutical carrier and a nucleic acid segment that encodes (a) a polypeptide having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a peptide that is a fragment of SEQ ID NO: 1; (c) a conservative variant of (a) or (b); or an expression cassette according to claim 30.
35. The composition of claim 34, wherein the composition is formulated for treatment of SARS-CoV.
36. The composition of claim 34, wherein the composition is formulated for prevention of SARS-CoV fusion with, or entry into, mammalian cells.
37. A viral vaccine comprising a pharmaceutical carrier, a viral vector and a nucleic acid segment that encodes (a) a polypeptide having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a peptide that is a fragment of SEQ ID NO: 1; (c) a conservative variant of (a) or (b); or an expression cassette according to claim 30.
38. The viral vaccine according to claim 34, wherein the viral vaccine is formulated in unit dosage form.
39. A peptide vaccine comprising a pharmaceutical carrier and (a) a peptide having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a fragment of SEQ ID NO: 1; (c) a peptidomimetic of (a) or (b); (d) or a conservative variant of (a) or (b).
40. The peptide vaccine according to claim 39, wherein the peptide vaccine is formulated in unit dosage form.
41. A microorganism vaccine comprising a pharmaceutical carrier and a microorganism that expresses (a) a peptide having any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a fragment of SEQ ID NO: 1; or (c) a conservative variant of (a) or (b).

42. The microorganism vaccine according to claim 41, wherein the microorganism is selected from the group consisting of *Salmonella* and *Listeria monocytogenes*.
- 5
43. The microorganism vaccine according to claim 42, wherein the microorganism vaccine is formulated in unit dosage form.
44. A DNA vaccine comprising a pharmaceutical carrier and vector into which is inserted a nucleic acid segment that encodes (a) an amino acid sequence as put forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (b) a fragment of SEQ ID NO: 1; or (c) a conservative variant of (a) or (b).
- 10
45. The DNA vaccine according to claim 44, wherein the vector is selected from the group consisting of a plasmid, a cosmid, a yeast artificial chromosome, a bacterial artificial chromosome, an F-factor, a virus, and a phagemid.
- 15
46. The DNA vaccine according to claim 44, wherein the DNA vaccine is formulated in unit dosage form.
- 20
47. The DNA vaccine according to claim 46, wherein the DNA vaccine further comprises a myonecrotic agent.
- 25
48. The DNA vaccine according to claim 47, wherein the myonecrotic agent is bupivacaine or cardiotoxin.
49. An antibody that binds to an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
- 30

50. The antibody according to claim 49, wherein the antibody specifically binds to an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
- 5 51. The antibody according to claim 49, wherein the antibody specifically binds to a S protein receptor binding domain.
52. The antibody according to claim 49, wherein the antibody is a
monoclonal antibody, a polyclonal antibody, a single-chain antibody, an
10 antigen-binding antibody fragment, or a humanized antibody.
53. The antibody according to claim 52, wherein the antigen-binding
antibody fragment is an scFv, Fv, Fab', Fab, diabody, linear antibody or
F(ab')₂.
- 15 54. The antibody according to claim 49, wherein the antibody is coupled to a detectable tag.
55. The antibody according to claim 54, wherein the detectable tag is a
20 fluorescent protein, a fluorescent marker, a radiolabel, an enzyme, or an affinity tag.
56. The antibody according to claim 49, wherein the antibody is coupled to a
toxin.
- 25 57. The antibody according to claim 56, wherein the toxin is an A chain
toxin, a ribosome inactivating protein, α -sarcin, gelonin, aspergillin,
ricin, a ribonuclease, an epipodophyllotoxin, diphtheria toxin,
Pseudomonas exotoxin, ricin, doxorubicin, daunorubicin, taxol, ethidium
30 bromide, mitomycin, etoposide, teniposide, vincristine, vinblastine,
colchicine, dihydroxy anthracin dione, actinomycin D, PE40, abrin, or a
glucocorticoid.

58. A pharmaceutical composition comprising a pharmaceutical carrier and an antibody that binds to an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
- 5
59. A method to immunize a mammal against severe acute respiratory syndrome comprising administering to the mammal a therapeutically effective amount of an antibody that binds to an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a
- 10 fragment of SEQ ID NO: 1.
60. The method of claim 59, wherein the antibody specifically binds to an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
- 15
61. The method of claim 59, wherein the mammal is a human.
62. A method to treat severe acute respiratory syndrome in a mammal comprising administering to the mammal a therapeutically effective
- 20 amount of an antibody that binds to an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
63. The method of claim 62, wherein the antibody specifically binds to an
- 25 amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
64. The method of claim 62, wherein the mammal is a human.
- 30 65. The method of claim 59 or 62, wherein the antibody is formulated with a pharmaceutical carrier or diluent.
66. A method for treating or inhibiting severe acute respiratory syndrome in a mammal comprising administering to the mammal a therapeutically

effective amount of a S polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.

- 5 67. A method for raising an immune response in a mammal against a SARS coronavirus spike protein comprising administering a therapeutically effective amount of a polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
- 10 68. The method of claim 67, wherein the polypeptide comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 25, 34, 51, 52, 56, 57, 58, 59, 61, 62, 63; or a fragment of SEQ ID NO: 1.
- 15 69. The method of claim 67, wherein the mammal is a human.
70. A method to diagnose severe acute respiratory syndrome in an animal comprising:
- 20 (a) contacting a biological sample obtained from the animal with an antibody that binds to an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1; and
- (b) determining if the antibody binds to the biological sample.
- 25 71. The method of claim 70, wherein the animal is a mammal.
72. The method of claim 70, wherein the mammal is a human.
- 30 73. A method for making an antibody comprising: obtaining an animal that was immunized with (a) a peptide fragment of a polypeptide having an amino acid sequence as set forth in SEQ ID NO: 1; (b) a polypeptide having an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; (c) a peptidemimetic of (a) or (b), or (d) a

- conservative variant of (a) or (b); and isolating an antibody that binds to (a).
74. A method to make an antibody comprising: obtaining an animal that was immunized with a coupled protein having a carrier protein coupled to (a) a peptide fragment of a polypeptide having an amino acid sequence as set forth in SEQ ID NO: 1; (b) a polypeptide having an amino acid sequence as set forth in any one of SEQ ID NOs: 1, 13, 14, 15, 20-55; (c) a peptidemimetic of (a) or (b), or (d) a conservative variant of (a) or (b); and isolating an antibody that binds to a polypeptide having an amino acid sequence as set forth in SEQ ID NO:1.
75. A kit comprising packaging material and an antibody or aptamer that binds to an amino acid sequence as set forth in any one of SEQ ID NOs: 1, 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
76. The kit of claim 75, wherein the antibody is formulated with a pharmaceutical carrier or diluent.
77. The kit of claim 75, further comprising a syringe.
78. A kit comprising packaging material and a therapeutically effective amount of a S polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
79. The kit of claim 78, wherein the S polypeptide is formulated with a pharmaceutical carrier or diluent.
80. The kit of claim 78, further comprising a syringe.
81. A monoclonal antibody that specifically binds to an amino acid sequence as set forth in any one of SEQ ID NOs:1, 13, 14, 15, 20-59, 61-63.

82. An isolated polyclonal antibody that specifically binds to an amino acid sequence as set forth in any one of SEQ ID NOs:1, 13, 14, 15, 20-59, 61-63.
- 5 83. An aptamer that binds to an amino acid sequence as set forth in any one of SEQ ID NOs:1, 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.
- 10 84. A pharmaceutical composition comprising a pharmaceutical carrier and an aptamer that binds to an amino acid sequence as set forth in any one of SEQ ID NOs:1, 13, 14, 15, 20-59, 61-63; or a fragment of SEQ ID NO: 1.

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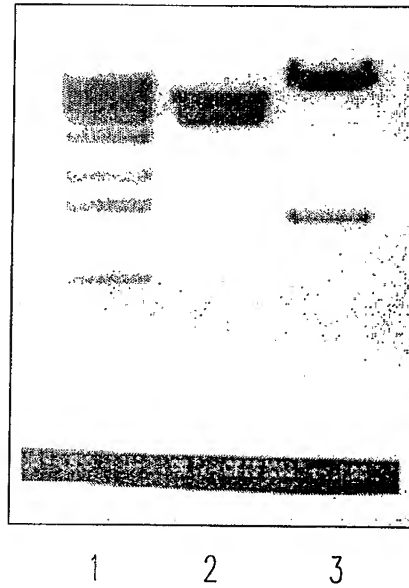


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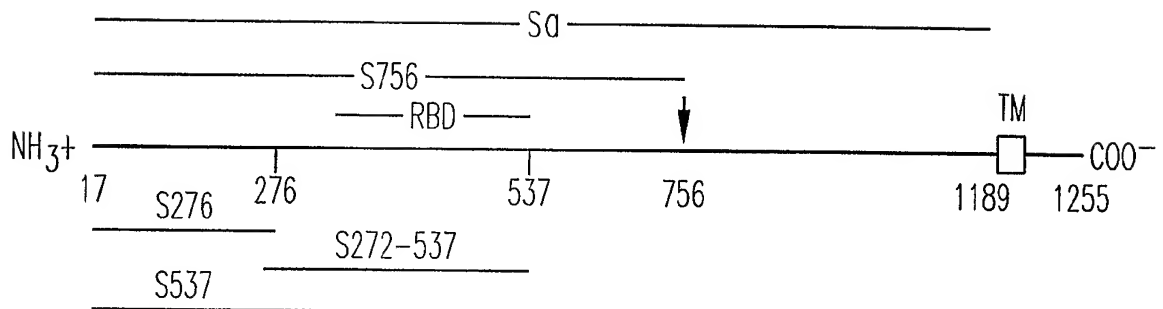


Fig. 1B

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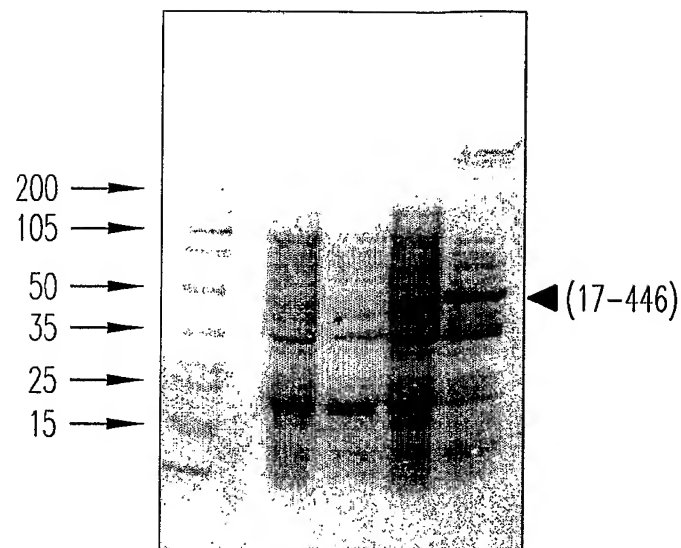


Fig. 2

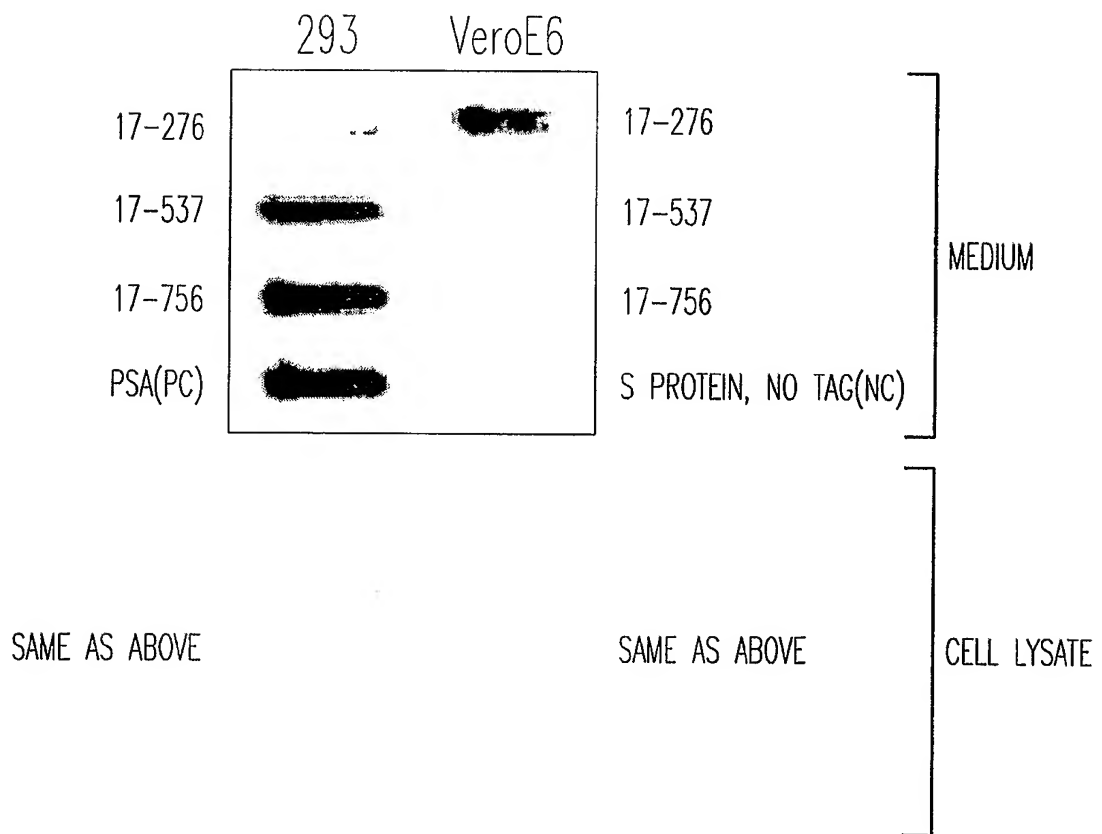


Fig. 3

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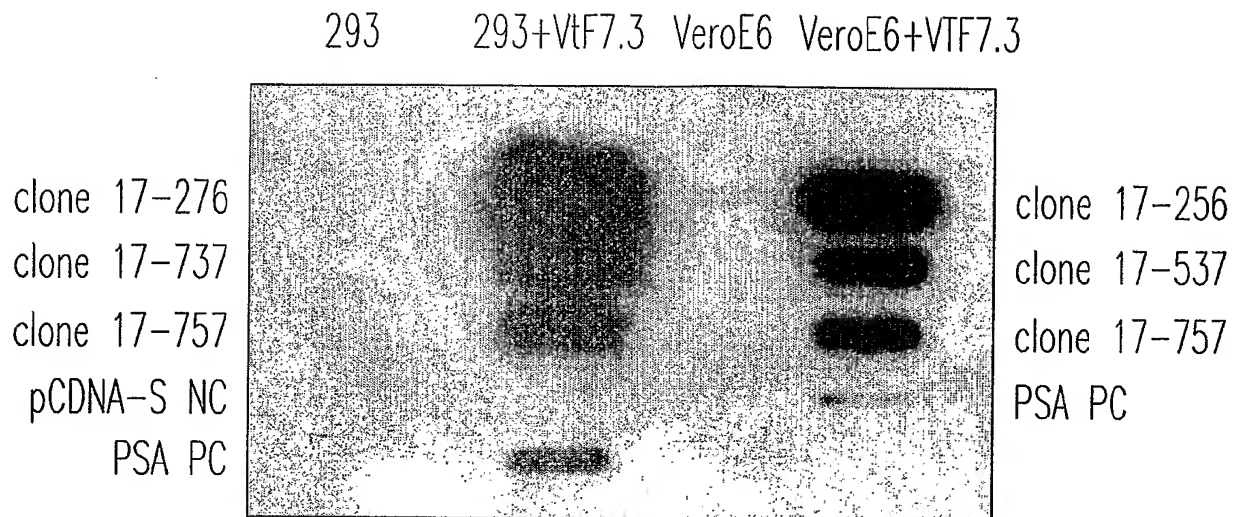


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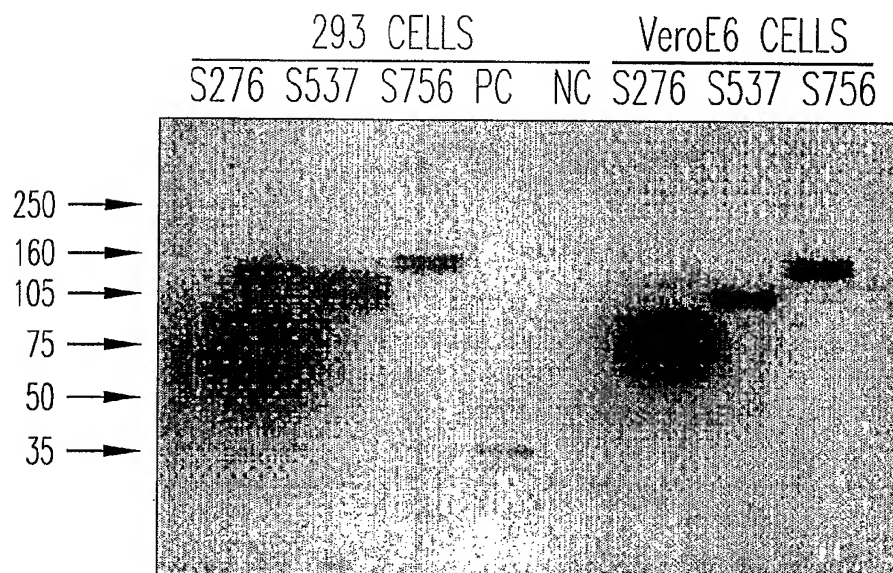


Fig. 4B

4/16

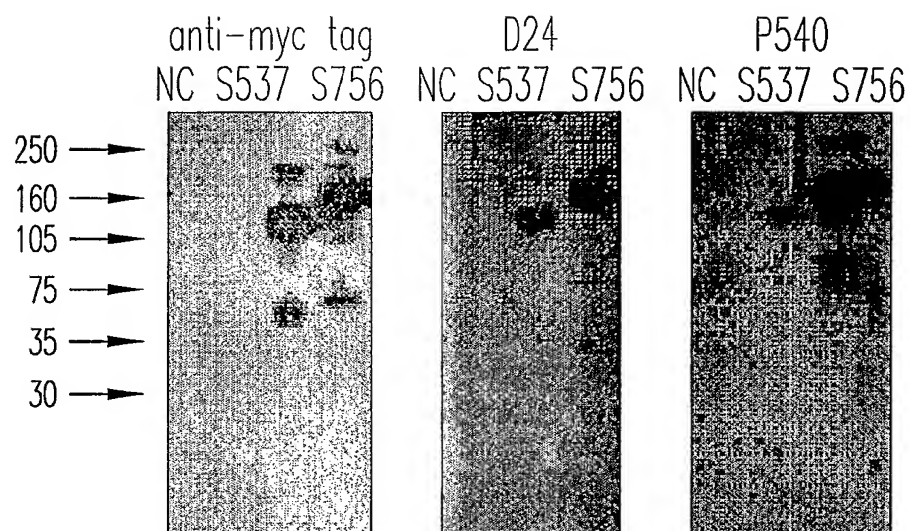


Fig. 4C

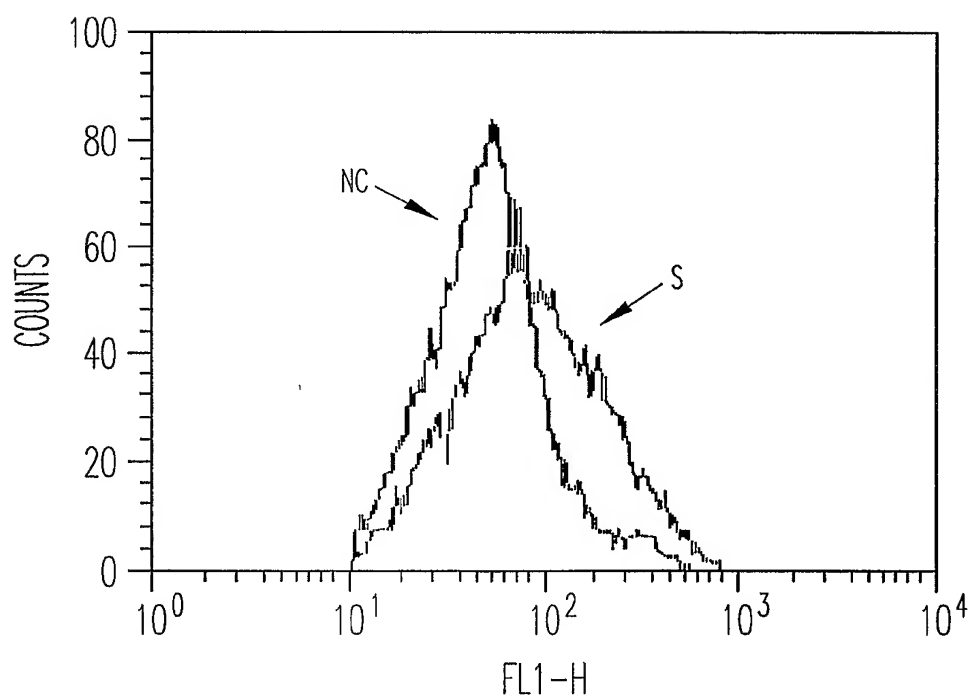


Fig. 5

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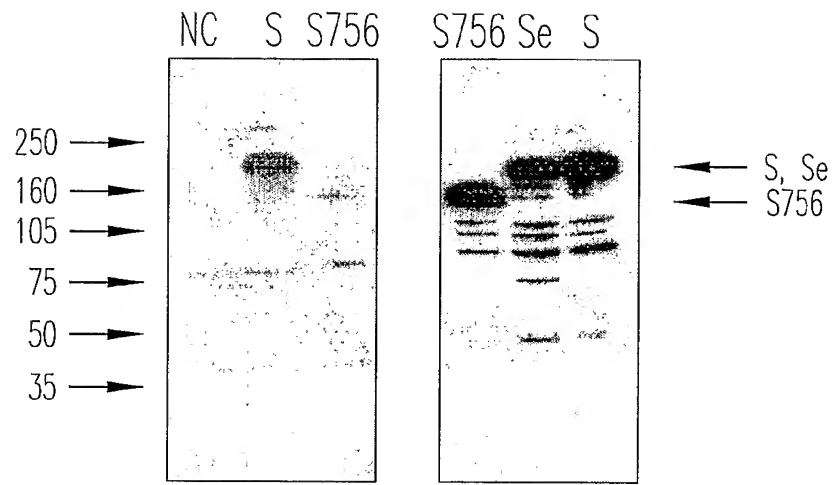


Fig. 6A

Fig. 6B

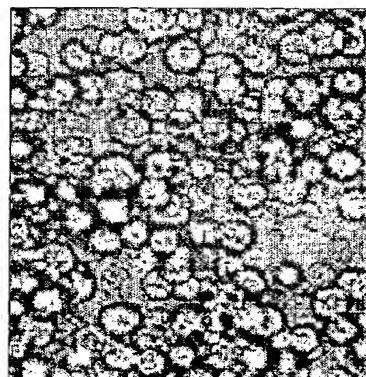


Fig. 7A

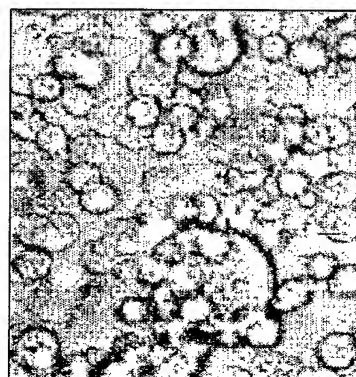


Fig. 7B

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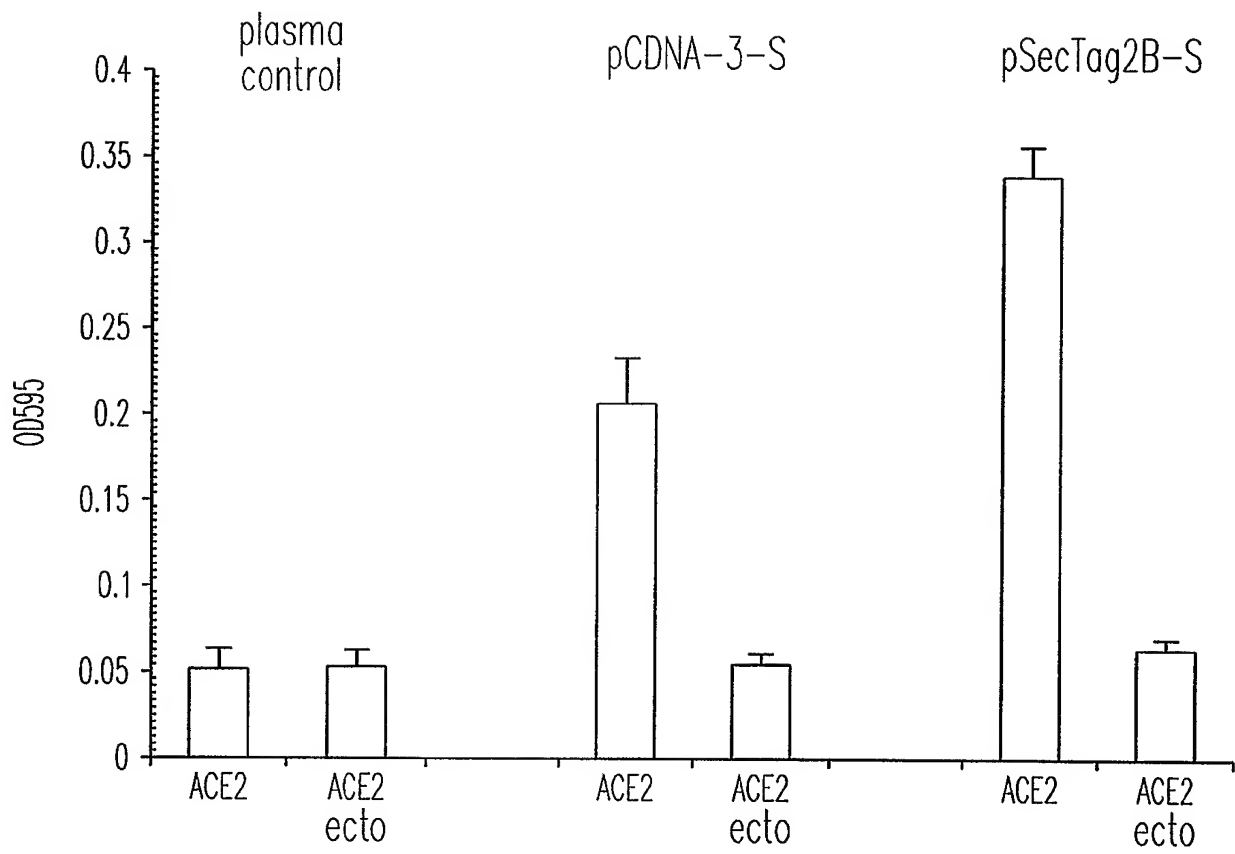


Fig. 7C

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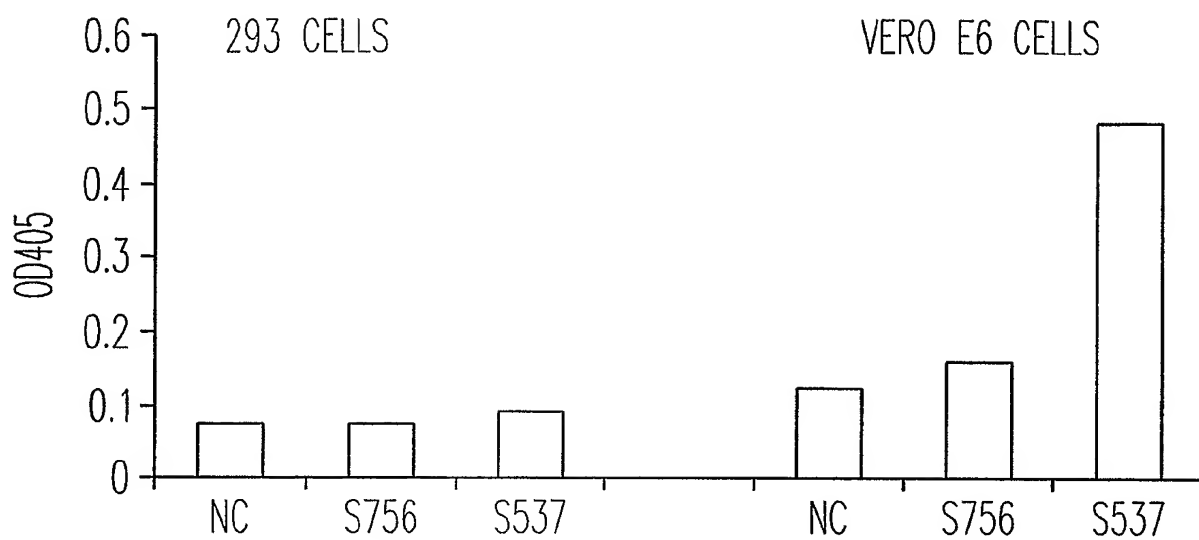


Fig. 8A

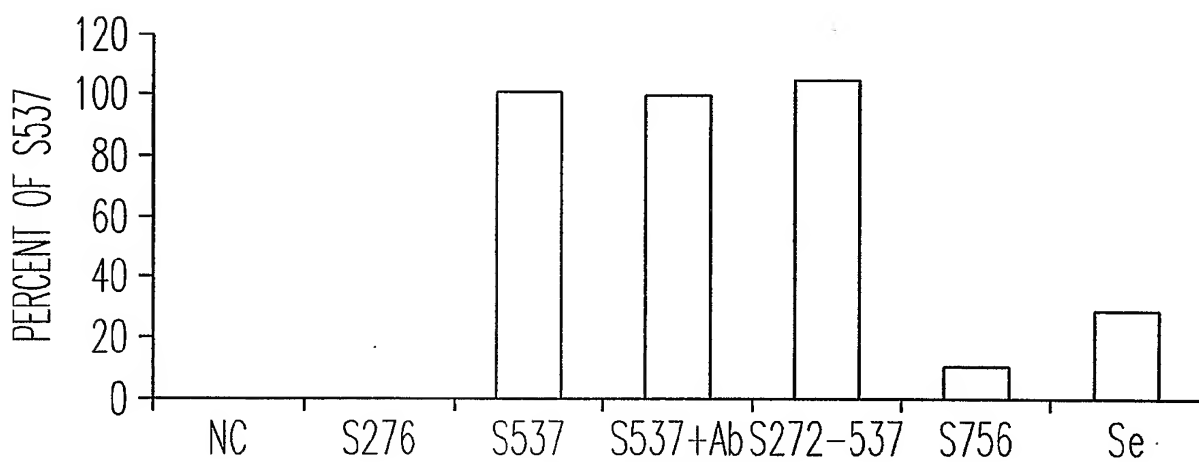


Fig. 8B

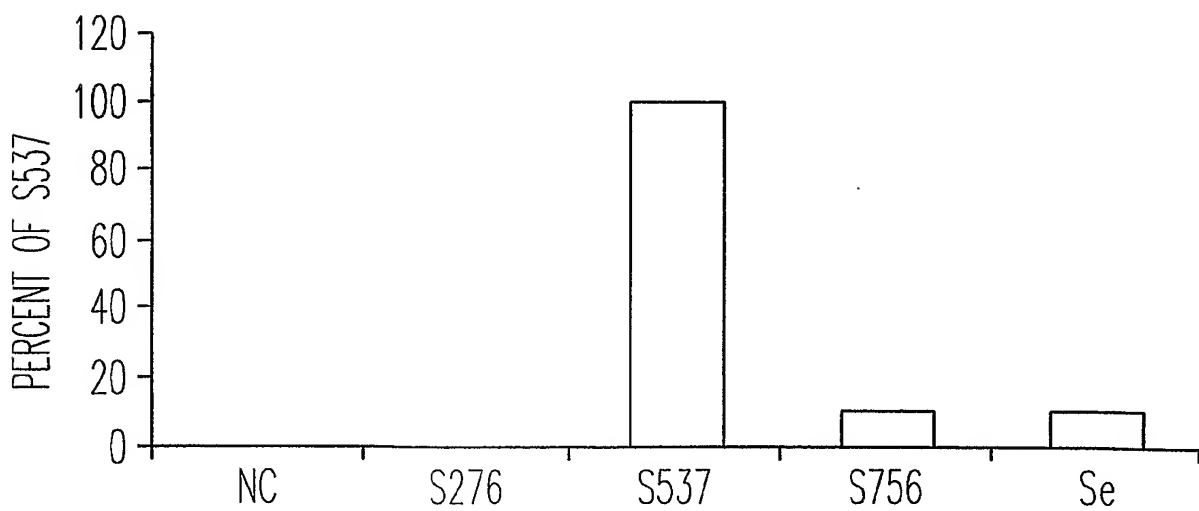


Fig. 8C

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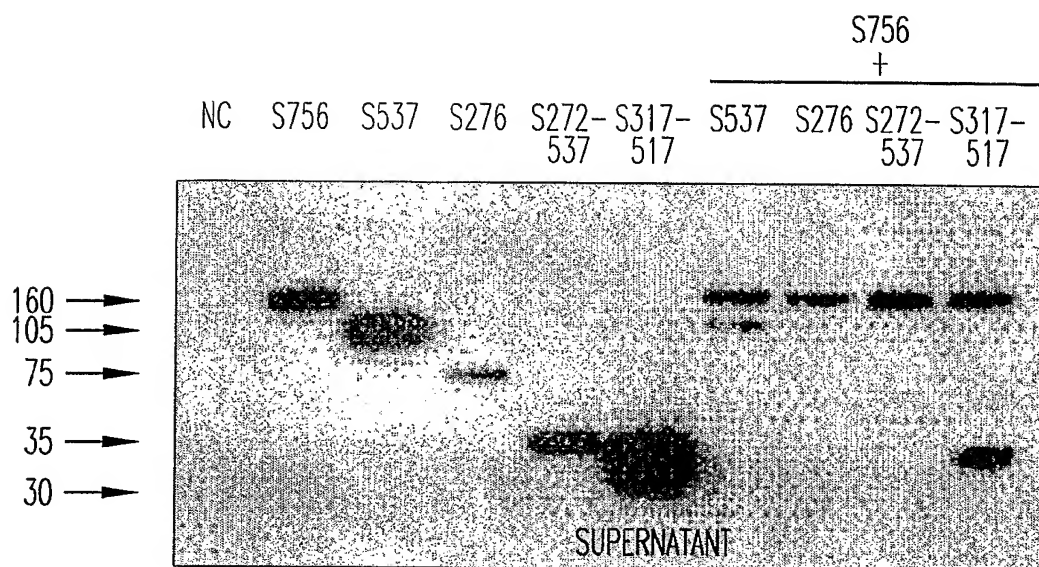


Fig. 9A

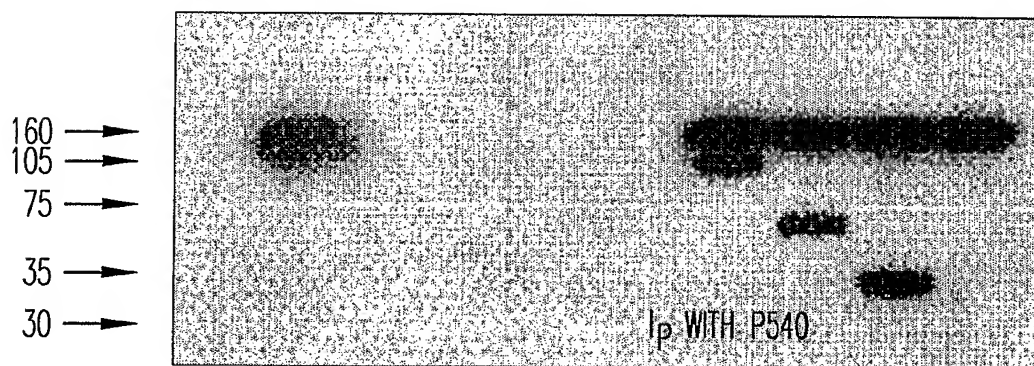


Fig. 9B

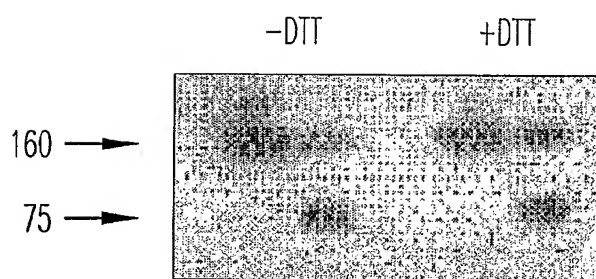


Fig. 9C

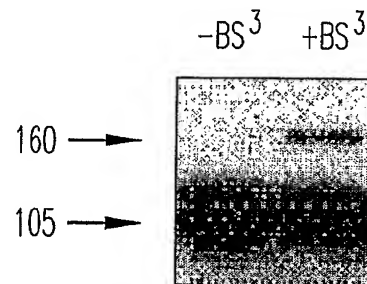


Fig. 9D

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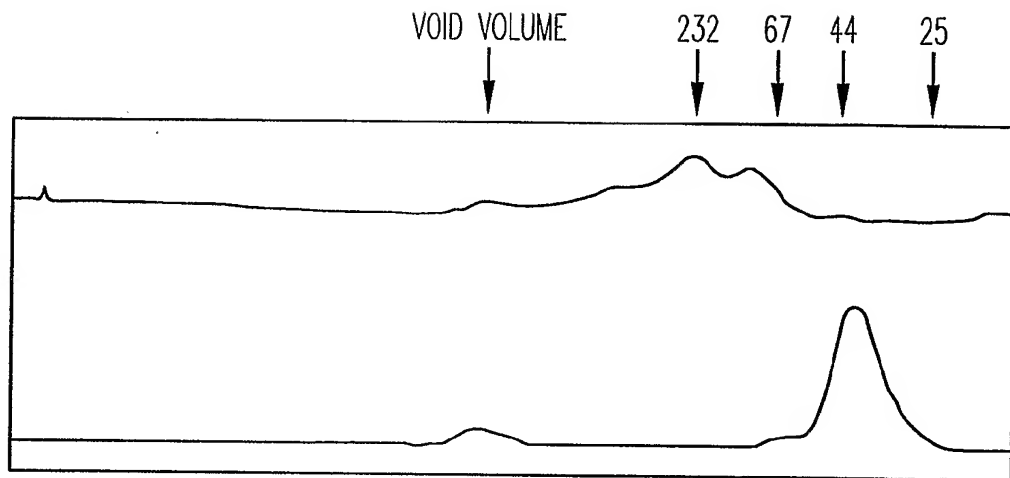


Fig. 10A

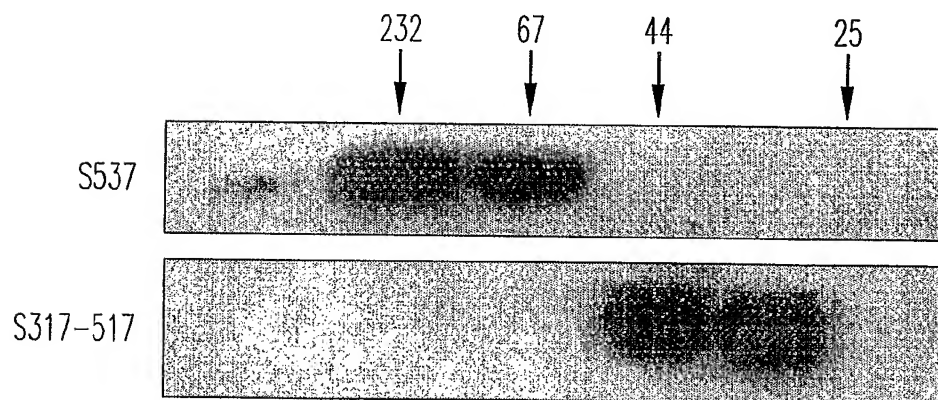


Fig. 10B

	S CONSTRUCTS	FUSION ACTIVITY
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Del1(103-1255)		-
Del2(311-1255)		-

Fig. 11A

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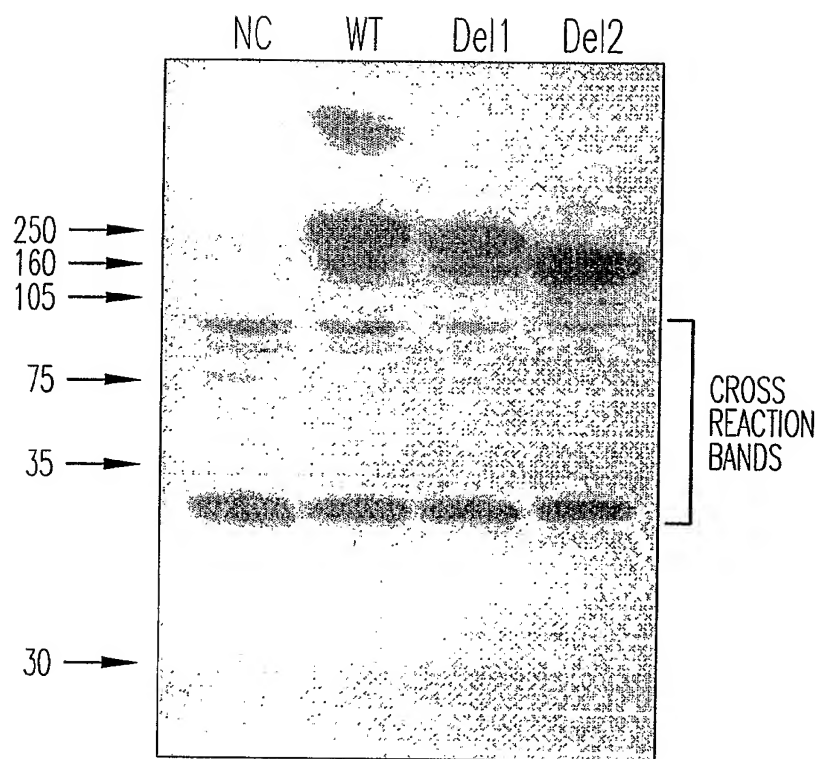


Fig. 11B

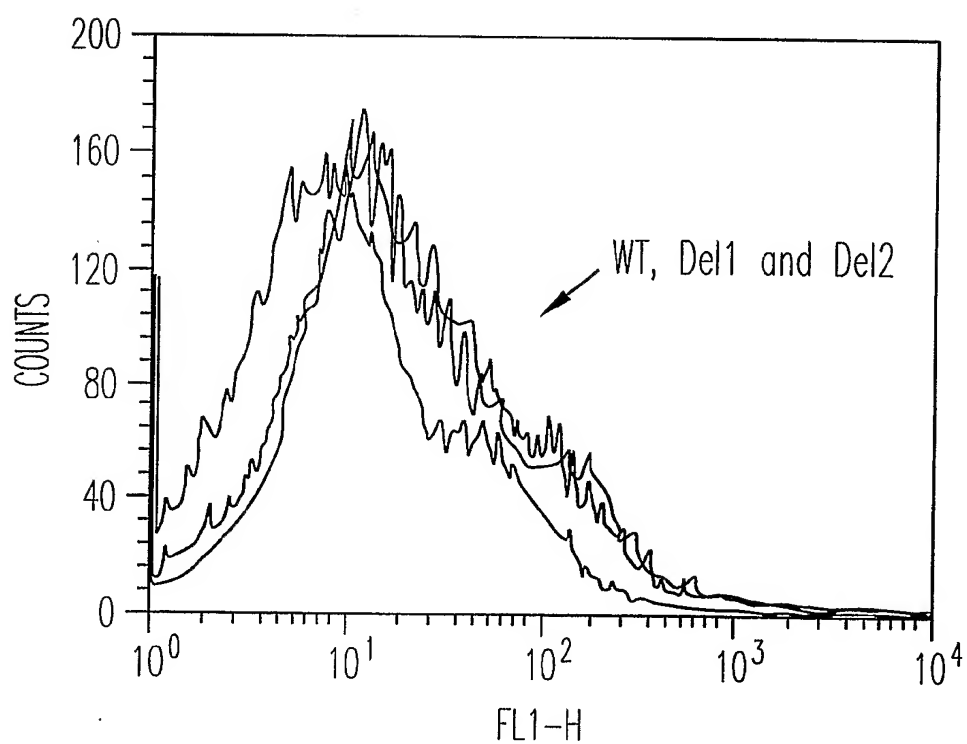


Fig. 11C

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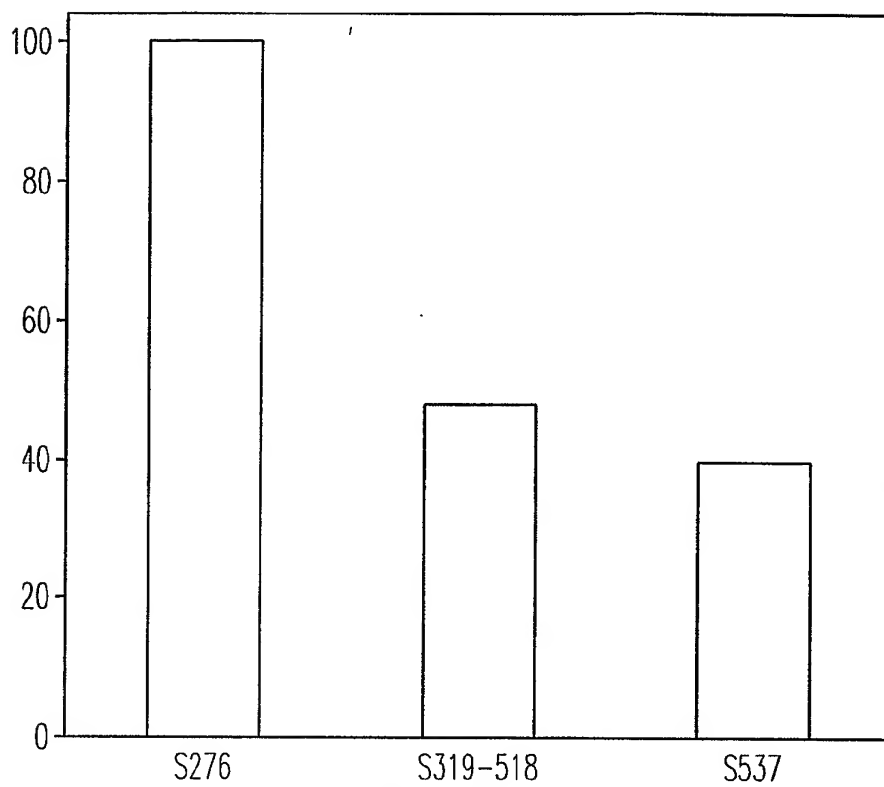


Fig. 12A

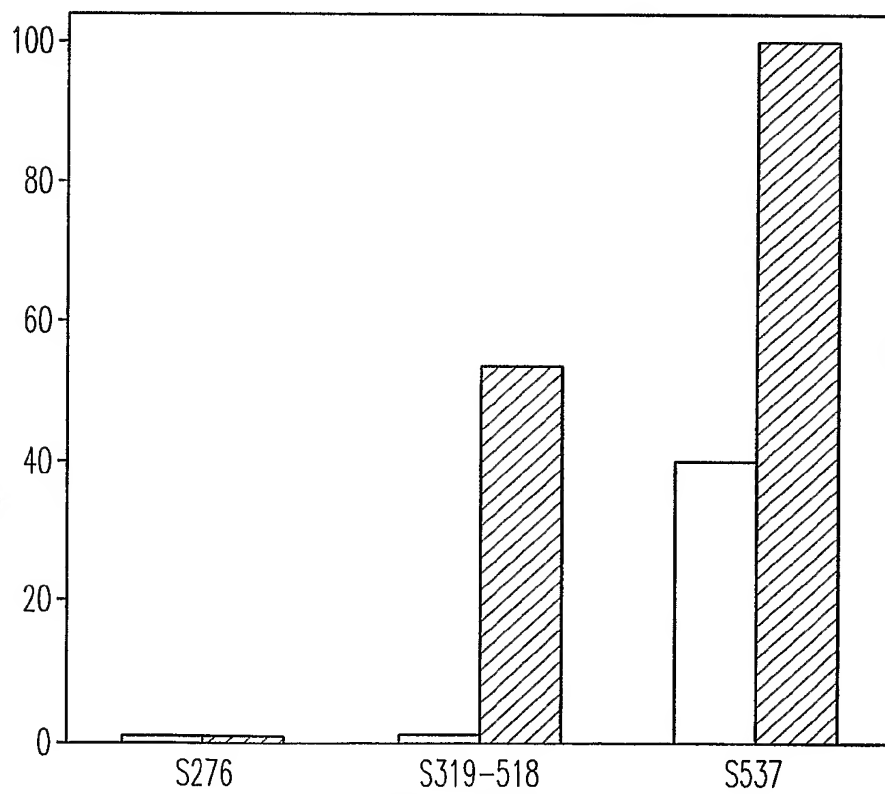


Fig. 12B

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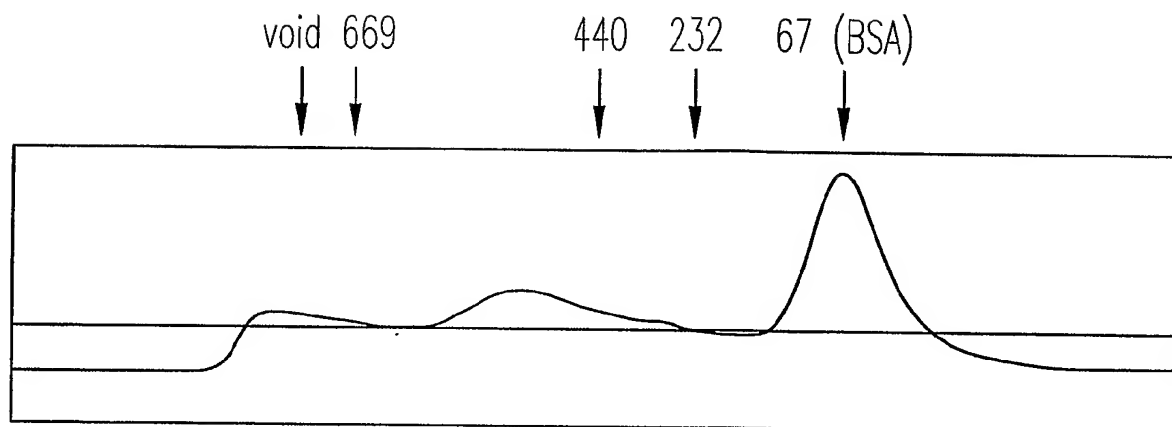


Fig. 13A

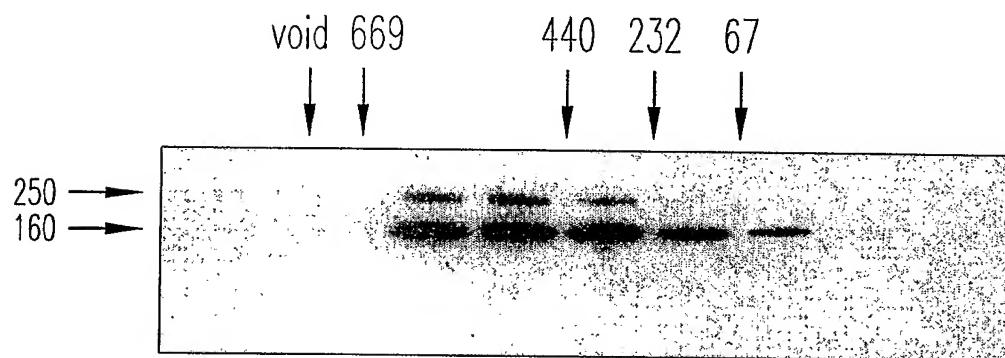


Fig. 13B

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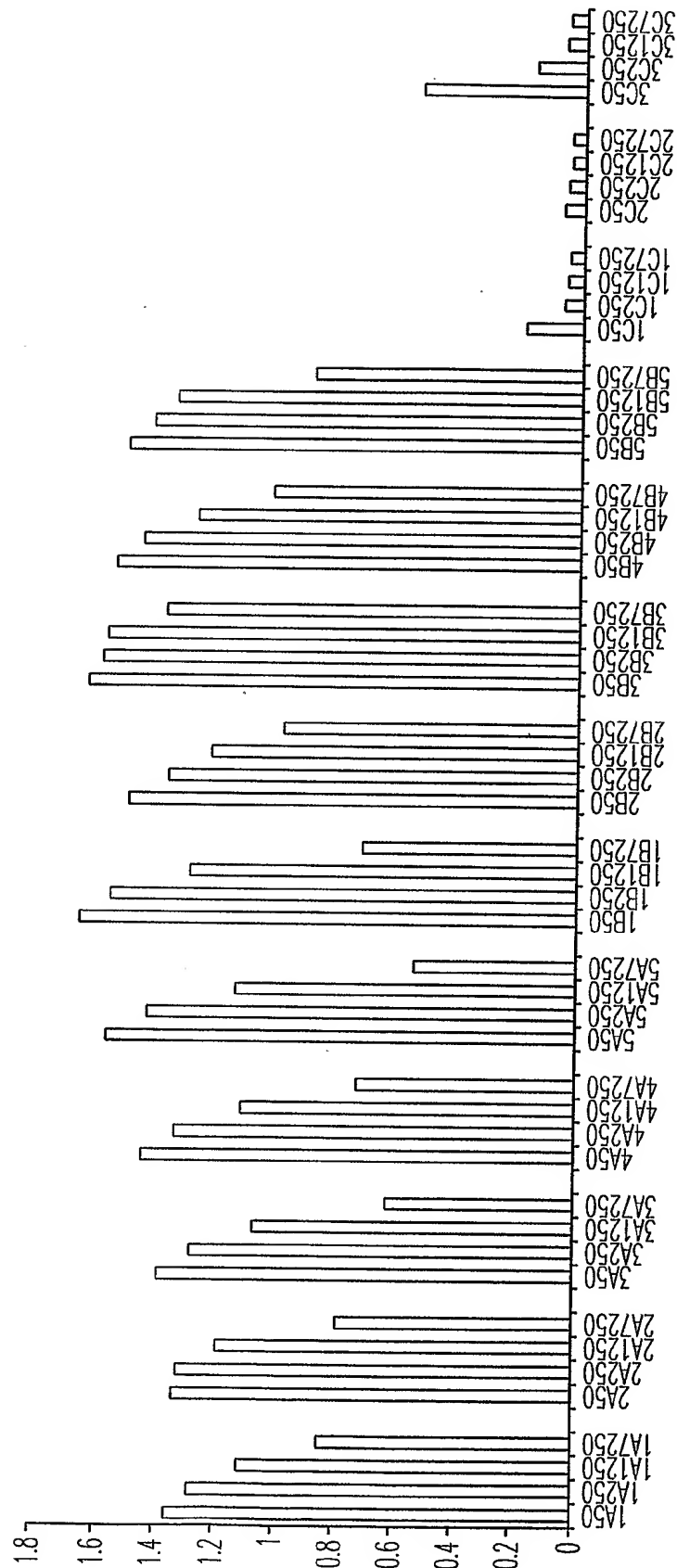


Fig. 14A

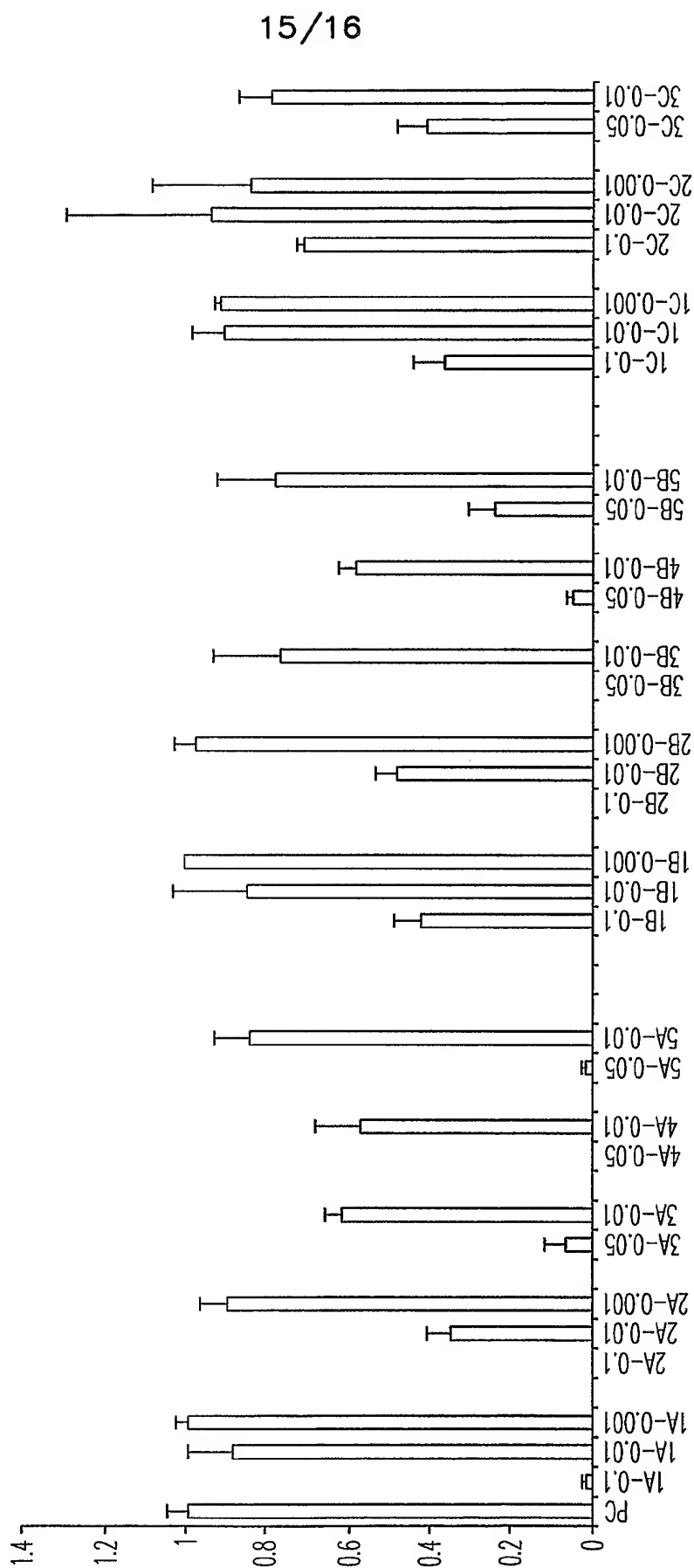


Fig. 14B

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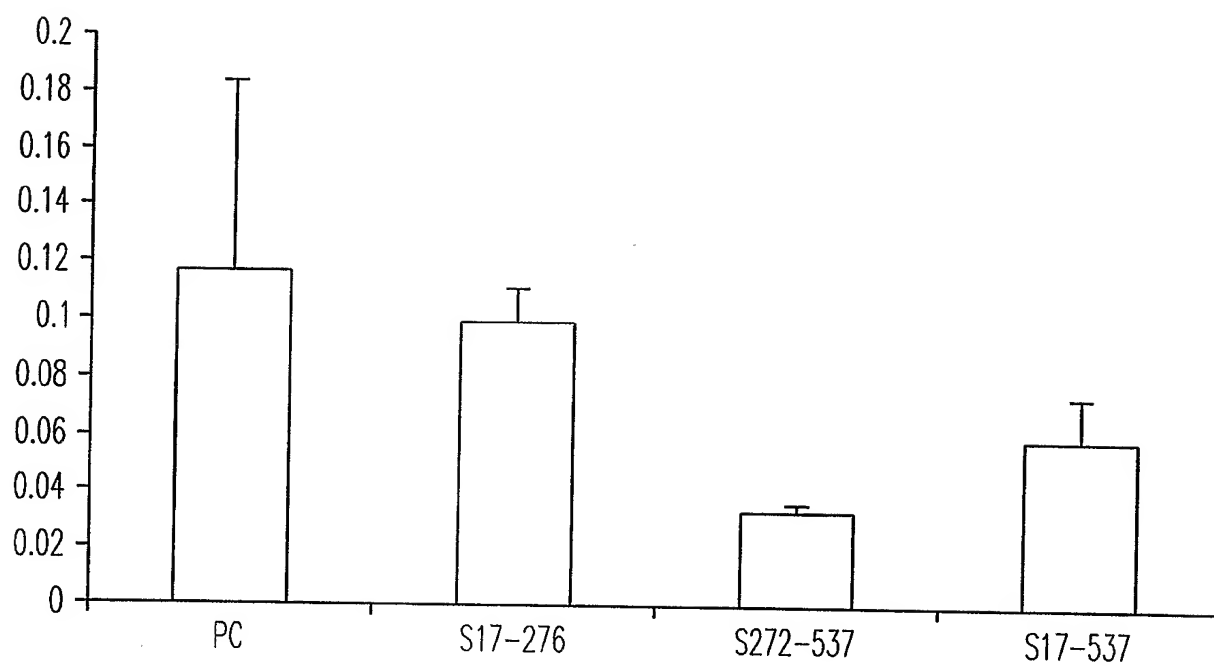


Fig. 15

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28

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 35 40 45
 5Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 50 55 60
 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 65 70 75 80
 Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
 10 85 90 95
 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 100 105 110
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 115 120 125
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 130 135 140
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 145 150 155 160
 Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 20 165 170 175
 Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
 180 185 190
 Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
 195 200 205
 25Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 210 215 220
 Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 225 230 235 240
 Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 30 245 250 255
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 260 265 270
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 275 280 285
 35Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 290 295 300
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 305 310 315 320
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 40 325 330 335
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 340 345 350

11

Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
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 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 370 375 380
 5Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 385 390 395 400
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 405 410 415
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 10 420 425 430
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 435 440 445
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 450 455 460
 15Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 465 470 475 480
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 485 490 495
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 20 500 505 510
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 515 520 525
 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 530 535 540
 25Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
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 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 565 570 575
 Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
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 Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
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 Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
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 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
 645 650 655
 Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
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 Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
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12

Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
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<211> 429

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<213> SARS coronavirus

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 35 40 45
 Leu Ala Asp Ala Gly Phe Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp
 50 55 60
 Ile Asn Ala Arg Asp Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr
 2565 70 75 80
 Val Leu Pro Pro Leu Leu Thr Asp Asp Met Ile Ala Ala Tyr Thr Ala
 85 90 95
 Ala Leu Val Ser Gly Thr Ala Thr Ala Gly Trp Thr Phe Gly Ala Gly
 100 105 110
 30Ala Ala Leu Gln Ile Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn
 115 120 125
 Gly Ile Gly Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln Ile
 130 135 140
 Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr
 35145 150 155 160
 Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn
 165 170 175
 Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly
 180 185 190
 40Ala Ile Ser Ser Val Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val
 195 200 205

13

Glu Ala Glu Val Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser
 210 215 220
 Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg
 225 230 235 240
 5Ala Ser Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly
 245 250 255
 Gln Ser Lys Arg Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser
 260 265 270
 Phe Pro Gln Ala Ala Pro His Gly Val Val Phe Leu His Val Thr Tyr
 10 275 280 285
 Val Pro Ser Gln Glu Arg Asn Phe Thr Thr Ala Pro Ala Ile Cys His
 290 295 300
 Glu Gly Lys Ala Tyr Phe Pro Arg Glu Gly Val Phe Val Phe Asn Gly
 305 310 315 320
 15Thr Ser Trp Phe Ile Thr Gln Arg Asn Phe Phe Ser Pro Gln Ile Ile
 325 330 335
 Thr Thr Asp Asn Thr Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly
 340 345 350
 Ile Ile Asn Asn Thr Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser
 20 355 360 365
 Phe Lys Glu Glu Leu Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp
 370 375 380
 Val Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile
 385 390 395 400
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<212> PRT

<213> SARS coronavirus

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 35 40 45

14

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 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 65 70 75 80
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 85 90 95
 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 100 105 110
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 10 115 120 125
 Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 130 135 140
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 145 150 155 160
 15Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 165 170 175
 Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
 180 185 190
 Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
 20 195 200 205
 Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 210 215 220
 Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 225 230 235 240
 25Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 245 250 255
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 260 265 270
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 30 275 280 285
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 290 295 300
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 305 310 315 320
 35Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 325 330 335
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 340 345 350
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 40 355 360 365
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 370 375 380

15

Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 385 390 395 400
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 405 410 415
 5Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
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 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 435 440 445
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
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 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 465 470 475 480
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 485 490 495
 15Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 500 505 510
 Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 515 520 525
 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 20 530 535 540
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 545 550 555 560
 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 565 570 575
 25Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
 580 585 590
 Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
 595 600 605
 Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
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 His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
 625 630 635 640
 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
 645 650 655
 35Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
 660 665 670
 Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
 675 680 685
 Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
 40 690 695 700
 Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
 705 710 715 720

16

Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
 725 730 735
 Ala Ala Glu Gln Asp Glu Val Phe Ala Gln Val Lys Gln Met Tyr Lys
 740 745 750
 5Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe Ser Gln Ile Leu
 755 760 765
 Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile Glu Asp Leu Leu
 770 775 780
 Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met Lys Gln Tyr Gly
 10785 790 795 800
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 835 840 845
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 Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn Val Leu Tyr Glu
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 Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp
 900 905 910
 25Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu
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 Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn Asp Ile Leu Ser
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 Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp Arg Leu Ile Thr
 30945 950 955 960
 Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg
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 980 985 990
 35Glu Cys Val Leu Gly Gln Ser Lys Arg Val Asp Phe Cys Gly Lys Gly
 995 1000 1005
 Tyr His Leu Met Ser Phe Pro Gln Ala Ala Pro His Gly Val Val Phe
 1010 1015 1020
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 Pro Ala Ile Cys His Glu Gly Lys Ala Tyr Phe Pro Arg Glu Gly Val
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17

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 1075 1080 1085
 5Asp Val Val Ile Gly Ile Ile Asn Asn Thr Val Tyr Asp Pro Leu Gln
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 1105 1110 1115 1120
 His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala
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 15Glu Gln
 1170

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18

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24

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25<211> 100

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<213> SARS coronavirus

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Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln

20

25

30

His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg

35

35

40

45

Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser

50

55

60

Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val

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70

75

80

40Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn

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90

95

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Val Val Arg Gly
100

<210> 21

5<211> 100

<212> PRT

<213> SARS coronavirus

<400> 21

10Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln Ser Val Ile Ile
1 5 10 15
Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys Asn Phe Glu Leu
20 25 30
Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met Gly Thr Gln Thr
15 35 40 45
His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr Phe Glu Tyr Ile
50 55 60
Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser Gly Asn Phe Lys
65 70 75 80
20His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly Phe Leu Tyr Val
85 90 95
Tyr Lys Gly Tyr
100

25<210> 22

<211> 100

<212> PRT

<213> SARS coronavirus

30<400> 22

Gln Pro Ile Asp Val Val Arg Asp Leu Pro Ser Gly Phe Asn Thr Leu
1 5 10 15
Lys Pro Ile Phe Lys Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe Arg
20 25 30
35Ala Ile Leu Thr Ala Phe Ser Pro Ala Gln Asp Ile Trp Gly Thr Ser
35 40 45
Ala Ala Ala Tyr Phe Val Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu
50 55 60
Lys Tyr Asp Glu Asn Gly Thr Ile Thr Asp Ala Val Asp Cys Ser Gln
4065 70 75 80
Asn Pro Leu Ala Glu Leu Lys Cys Ser Val Lys Ser Phe Glu Ile Asp
85 90 95

20

Lys Gly Ile Tyr
100

<210> 23

5<211> 100

<212> PRT

<213> SARS coronavirus

<400> 23

10Gln Thr Ser Asn Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe
1 5 10 15
Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr
20 25 30
Lys Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys
15 35 40 45
Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe
50 55 60
Lys Cys Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser
65 70 75 80
20Asn Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln
85 90 95
Ile Ala Pro Gly
100

25<210> 24

<211> 100

<212> PRT

<213> SARS coronavirus

30<400> 24

Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
1 5 10 15
Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
20 25 30
35Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
35 40 45
Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
50 55 60
Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
4065 70 75 80
Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
85 90 95

21

Val Val Leu Ser
100

<210> 25

5<211> 100

<212> PRT

<213> SARS coronavirus

<400> 25

10Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly Pro Lys Leu Ser
1 5 10 15
Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn Phe Asn Gly Leu
20 25 30
Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg Phe Gln Pro Phe
15 35 40 45
Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp Ser Val Arg Asp
50 55 60
Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys Ala Phe Gly Gly
65 70 75 80
20Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser Glu Val Ala Val
85 90 95
Leu Tyr Gln Asp
100

25<210> 26

<211> 100

<212> PRT

<213> SARS coronavirus

30<400> 26

Val Asn Cys Thr Asp Val Ser Thr Ala Ile His Ala Asp Gln Leu Thr
1 5 10 15
Pro Ala Trp Arg Ile Tyr Ser Thr Gly Asn Asn Val Phe Gln Thr Gln
20 25 30
35Ala Gly Cys Leu Ile Gly Ala Glu His Val Asp Thr Ser Tyr Glu Cys
35 40 45
Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala Ser Tyr His Thr Val Ser
50 55 60
Leu Leu Arg Ser Thr Ser Gln Lys Ser Ile Val Ala Tyr Thr Met Ser
4065 70 75 80
Leu Gly Ala Asp Ser Ser Ile Ala Tyr Ser Asn Asn Thr Ile Ala Ile
85 90 95

22

Pro Thr Asn Phe
100

<210> 27

5<211> 100

<212> PRT

<213> SARS coronavirus

<400> 27

10Ser Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr
1 5 10 15
Ser Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala
20 25 30
Asn Leu Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala
15 35 40 45
Leu Ser Gly Ile Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe
50 55 60
Ala Gln Val Lys Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly
65 70 75 80
20Gly Phe Asn Phe Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys
85 90 95
Arg Ser Phe Ile
100

25<210> 28

<211> 100

<212> PRT

<213> SARS coronavirus

30<400> 28

Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
1 5 10 15
Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
20 25 30
35Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
35 40 45
Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
50 55 60
Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
4065 70 75 80
Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
85 90 95

23

Val Leu Tyr Glu
100

<210> 29

5<211> 100

<212> PRT

<213> SARS coronavirus

<400> 29

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10Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile
   1             5             10             15
   Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp
           20             25             30
   Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu Val Lys Gln Leu
15           35             40             45
   Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn Asp Ile Leu Ser
           50             55             60
   Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp Arg Leu Ile Thr
   65           70             75             80
20Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg
           85             90             95
   Ala Ala Glu Ile
           100

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25<210> 30

<211> 100

<212> PRT

<213> SARS coronavirus

30<400> 30

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   Arg Ala Ser Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu
   1             5             10             15
   Gly Gln Ser Lys Arg Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met
           20             25             30
35Ser Phe Pro Gln Ala Ala Pro His Gly Val Val Phe Leu His Val Thr
           35             40             45
   Tyr Val Pro Ser Gln Glu Arg Asn Phe Thr Thr Ala Pro Ala Ile Cys
           50             55             60
   His Glu Gly Lys Ala Tyr Phe Pro Arg Glu Gly Val Phe Val Phe Asn
4065           70             75             80
   Gly Thr Ser Trp Phe Ile Thr Gln Arg Asn Phe Phe Ser Pro Gln Ile
           85             90             95

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24

Ile Thr Thr Asp
100

<210> 31

5<211> 90

<212> PRT

<213> SARS coronavirus

<400> 31

10Asn Thr Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Ile Asn
1 5 10 15
Asn Thr Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu
20 25 30
Glu Leu Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu
15 35 40 45
Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu
50 55 60
Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile
65 70 75 80
20Asp Leu Gln Glu Leu Gly Lys Tyr Glu Gln
85 90

<210> 32

<211> 200

25<212> PRT

<213> SARS coronavirus

<400> 32

Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
30 1 5 10 15
Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
20 25 30
His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
35 40 45
35Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
50 55 60
Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
65 70 75 80
Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
40 85 90 95
Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
100 105 110

25

Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 115 120 125
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 130 135 140
 5Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 145 150 155 160
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 165 170 175
 Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 10 180 185 190
 Phe Leu Tyr Val Tyr Lys Gly Tyr
 195 200

<210> 33

15<211> 200

<212> PRT

<213> SARS coronavirus

<400> 33

20Gln Pro Ile Asp Val Val Arg Asp Leu Pro Ser Gly Phe Asn Thr Leu
 1 5 10 15
 Lys Pro Ile Phe Lys Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe Arg
 20 25 30
 Ala Ile Leu Thr Ala Phe Ser Pro Ala Gln Asp Ile Trp Gly Thr Ser
 25 35 40 45
 Ala Ala Ala Tyr Phe Val Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu
 50 55 60
 Lys Tyr Asp Glu Asn Gly Thr Ile Thr Asp Ala Val Asp Cys Ser Gln
 65 70 75 80
 30Asn Pro Leu Ala Glu Leu Lys Cys Ser Val Lys Ser Phe Glu Ile Asp
 85 90 95
 Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val Val Pro Ser Gly Asp
 100 105 110
 Val Val Arg Phe Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val
 35 115 120 125
 Phe Asn Ala Thr Lys Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys
 130 135 140
 Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe
 145 150 155 160
 40Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp
 165 170 175

26

Leu Cys Phe Ser Asn Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp
 180 185 190
 Asp Val Arg Gln Ile Ala Pro Gly
 195 200
 5
 <210> 34
 <211> 200
 <212> PRT
 <213> SARS coronavirus
 10
 <400> 34
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 1 5 10 15
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 15 20 25 30
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 35 40 45
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 50 55 60
 20 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 65 70 75 80
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 85 90 95
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 25 100 105 110
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 115 120 125
 Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 130 135 140
 30 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 145 150 155 160
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 165 170 175
 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 35 180 185 190
 Glu Val Ala Val Leu Tyr Gln Asp
 195 200
 <210> 35
 40 <211> 200
 <212> PRT
 <213> SARS coronavirus

27

<400> 35

Val Asn Cys Thr Asp Val Ser Thr Ala Ile His Ala Asp Gln Leu Thr
 1 5 10 15
 Pro Ala Trp Arg Ile Tyr Ser Thr Gly Asn Asn Val Phe Gln Thr Gln
 5 20 25 30
 Ala Gly Cys Leu Ile Gly Ala Glu His Val Asp Thr Ser Tyr Glu Cys
 35 40 45
 Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala Ser Tyr His Thr Val Ser
 50 55 60
 10Leu Leu Arg Ser Thr Ser Gln Lys Ser Ile Val Ala Tyr Thr Met Ser
 65 70 75 80
 Leu Gly Ala Asp Ser Ser Ile Ala Tyr Ser Asn Asn Thr Ile Ala Ile
 85 90 95
 Pro Thr Asn Phe Ser Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser
 15 100 105 110
 Met Ala Lys Thr Ser Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser
 115 120 125
 Thr Glu Cys Ala Asn Leu Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln
 130 135 140
 20Leu Asn Arg Ala Leu Ser Gly Ile Ala Ala Glu Gln Asp Arg Asn Thr
 145 150 155 160
 Arg Glu Val Phe Ala Gln Val Lys Gln Met Tyr Lys Thr Pro Thr Leu
 165 170 175
 Lys Tyr Phe Gly Gly Phe Asn Phe Ser Gln Ile Leu Pro Asp Pro Leu
 25 180 185 190
 Lys Pro Thr Lys Arg Ser Phe Ile
 195 200

<210> 36

30<211> 200

<212> PRT

<213> SARS coronavirus

<400> 36

35Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
 1 5 10 15
 Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
 20 25 30
 Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
 40 35 40 45
 Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
 50 55 60

28

Thr	Ala	Gly	Trp	Thr	Phe	Gly	Ala	Gly	Ala	Ala	Leu	Gln	Ile	Pro	Phe	
65					70				75						80	
Ala	Met	Gln	Met	Ala	Tyr	Arg	Phe	Asn	Gly	Ile	Gly	Val	Thr	Gln	Asn	
				85					90					95		
5Val	Leu	Tyr	Glu	Asn	Gln	Lys	Gln	Ile	Ala	Asn	Gln	Phe	Asn	Lys	Ala	
			100					105					110			
	Ile	Ser	Gln	Ile	Gln	Glu	Ser	Leu	Thr	Thr	Thr	Ser	Thr	Ala	Leu	Gly
			115					120					125			
	Lys	Leu	Gln	Asp	Val	Val	Asn	Gln	Asn	Ala	Gln	Ala	Leu	Asn	Thr	Leu
10		130					135					140				
	Val	Lys	Gln	Leu	Ser	Ser	Asn	Phe	Gly	Ala	Ile	Ser	Ser	Val	Leu	Asn
	145					150				155						160
	Asp	Ile	Leu	Ser	Arg	Leu	Asp	Lys	Val	Glu	Ala	Glu	Val	Gln	Ile	Asp
				165						170					175	
15Arg	Leu	Ile	Thr	Gly	Arg	Leu	Gln	Ser	Leu	Gln	Thr	Tyr	Val	Thr	Gln	
			180					185						190		
	Gln	Leu	Ile	Arg	Ala	Ala	Glu	Ile								
		195						200								

20<210> 37

<211> 190

<212> PRT

<213> SARS coronavirus

25<400> 37

Arg	Ala	Ser	Ala	Asn	Leu	Ala	Ala	Thr	Lys	Met	Ser	Glu	Cys	Val	Leu
1				5					10					15	
Gly	Gln	Ser	Lys	Arg	Val	Asp	Phe	Cys	Gly	Lys	Gly	Tyr	His	Leu	Met
			20					25					30		
30Ser	Phe	Pro	Gln	Ala	Ala	Pro	His	Gly	Val	Val	Phe	Leu	His	Val	Thr
			35					40					45		
Tyr	Val	Pro	Ser	Gln	Glu	Arg	Asn	Phe	Thr	Thr	Ala	Pro	Ala	Ile	Cys
	50					55					60				
His	Glu	Gly	Lys	Ala	Tyr	Phe	Pro	Arg	Glu	Gly	Val	Phe	Val	Phe	Asn
3565					70					75					80
Gly	Thr	Ser	Trp	Phe	Ile	Thr	Gln	Arg	Asn	Phe	Phe	Ser	Pro	Gln	Ile
				85					90					95	
Ile	Thr	Thr	Asp	Asn	Thr	Phe	Val	Ser	Gly	Asn	Cys	Asp	Val	Val	Ile
			100					105					110		
40Gly	Ile	Ile	Asn	Asn	Thr	Val	Tyr	Asp	Pro	Leu	Gln	Pro	Glu	Leu	Asp
			115					120					125		

29

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Ser Phe Lys Glu Glu Leu Asp Lys Tyr Phe Lys Asn His Thr Ser Pro
  130                      135                      140
Asp Val Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn
  145                      150                      155                      160
5Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn
                      165                      170                      175
Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr Glu Gln
                      180                      185                      190

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10<210> 38

<211> 400

<212> PRT

<213> SARS coronavirus

15<400> 38

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Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
  1                      5                      10                      15
Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
                      20                      25                      30
20His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
                      35                      40                      45
Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
  50                      55                      60
Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
2565                      70                      75                      80
Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
                      85                      90                      95
Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
                      100                      105                      110
30Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
                      115                      120                      125
Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
  130                      135                      140
Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
35145                      150                      155                      160
Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
                      165                      170                      175
Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
                      180                      185                      190
40Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
                      195                      200                      205

```

30

Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
 210 215 220
 Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 225 230 235 240
 5Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 245 250 255
 Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 260 265 270
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 10 275 280 285
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 290 295 300
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 305 310 315 320
 15Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 325 330 335
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 340 345 350
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 20 355 360 365
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 370 375 380
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 385 390 395 400

25

<210> 39
 <211> 600
 <212> PRT
 <213> SARS coronavirus

30

<400> 39
 Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
 1 5 10 15
 Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
 35 20 25 30
 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 35 40 45
 Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 50 55 60
 40Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 65 70 75 80

31

Ile	Pro	Phe	Lys	Asp	Gly	Ile	Tyr	Phe	Ala	Ala	Thr	Glu	Lys	Ser	Asn		
				85					90						95		
Val	Val	Arg	Gly	Trp	Val	Phe	Gly	Ser	Thr	Met	Asn	Asn	Lys	Ser	Gln		
			100					105					110				
5Ser	Val	Ile	Ile	Ile	Asn	Asn	Ser	Thr	Asn	Val	Val	Ile	Arg	Ala	Cys		
			115					120					125				
Asn	Phe	Glu	Leu	Cys	Asp	Asn	Pro	Phe	Phe	Ala	Val	Ser	Lys	Pro	Met		
			130					135					140				
Gly	Thr	Gln	Thr	His	Thr	Met	Ile	Phe	Asp	Asn	Ala	Phe	Asn	Cys	Thr		
10145					150					155					160		
Phe	Glu	Tyr	Ile	Ser	Asp	Ala	Phe	Ser	Leu	Asp	Val	Ser	Glu	Lys	Ser		
				165					170					175			
Gly	Asn	Phe	Lys	His	Leu	Arg	Glu	Phe	Val	Phe	Lys	Asn	Lys	Asp	Gly		
			180					185						190			
15Phe	Leu	Tyr	Val	Tyr	Lys	Gly	Tyr	Gln	Pro	Ile	Asp	Val	Val	Arg	Asp		
			195					200					205				
Leu	Pro	Ser	Gly	Phe	Asn	Thr	Leu	Lys	Pro	Ile	Phe	Lys	Leu	Pro	Leu		
			210					215					220				
Gly	Ile	Asn	Ile	Thr	Asn	Phe	Arg	Ala	Ile	Leu	Thr	Ala	Phe	Ser	Pro		
20225					230					235					240		
Ala	Gln	Asp	Ile	Trp	Gly	Thr	Ser	Ala	Ala	Ala	Tyr	Phe	Val	Gly	Tyr		
				245					250						255		
Leu	Lys	Pro	Thr	Thr	Phe	Met	Leu	Lys	Tyr	Asp	Glu	Asn	Gly	Thr	Ile		
			260					265						270			
25Thr	Asp	Ala	Val	Asp	Cys	Ser	Gln	Asn	Pro	Leu	Ala	Glu	Leu	Lys	Cys		
			275					280						285			
Ser	Val	Lys	Ser	Phe	Glu	Ile	Asp	Lys	Gly	Ile	Tyr	Gln	Thr	Ser	Asn		
			290					295					300				
Phe	Arg	Val	Val	Pro	Ser	Gly	Asp	Val	Val	Arg	Phe	Pro	Asn	Ile	Thr		
30305					310					315					320		
Asn	Leu	Cys	Pro	Phe	Gly	Glu	Val	Phe	Asn	Ala	Thr	Lys	Phe	Pro	Ser		
				325					330					335			
Val	Tyr	Ala	Trp	Glu	Arg	Lys	Lys	Ile	Ser	Asn	Cys	Val	Ala	Asp	Tyr		
			340					345						350			
35Ser	Val	Leu	Tyr	Asn	Ser	Thr	Phe	Phe	Ser	Thr	Phe	Lys	Cys	Tyr	Gly		
			355					360					365				
Val	Ser	Ala	Thr	Lys	Leu	Asn	Asp	Leu	Cys	Phe	Ser	Asn	Val	Tyr	Ala		
			370					375					380				
Asp	Ser	Phe	Val	Val	Lys	Gly	Asp	Asp	Val	Arg	Gln	Ile	Ala	Pro	Gly		
40385					390					395					400		
Gln	Thr	Gly	Val	Ile	Ala	Asp	Tyr	Asn	Tyr	Lys	Leu	Pro	Asp	Asp	Phe		
				405					410						415		

32

Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 420 425 430
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 435 440 445
 5Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 450 455 460
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 465 470 475 480
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 10 485 490 495
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 500 505 510
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 515 520 525
 15Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 530 535 540
 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 545 550 555 560
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 20 565 570 575
 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 580 585 590
 Glu Val Ala Val Leu Tyr Gln Asp
 595 600

25

<210> 40
 <211> 800
 <212> PRT
 <213> SARS coronavirus

30

<400> 40
 Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
 1 5 10 15
 Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
 35 20 25 30
 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 35 40 45
 Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 50 55 60
 40Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 65 70 75 80

Ile	Pro	Phe	Lys	Asp	Gly	Ile	Tyr	Phe	Ala	Ala	Thr	Glu	Lys	Ser	Asn	
				85					90						95	
Val	Val	Arg	Gly	Trp	Val	Phe	Gly	Ser	Thr	Met	Asn	Asn	Lys	Ser	Gln	
			100					105					110			
5Ser	Val	Ile	Ile	Ile	Asn	Asn	Ser	Thr	Asn	Val	Val	Ile	Arg	Ala	Cys	
			115					120					125			
	Asn	Phe	Glu	Leu	Cys	Asp	Asn	Pro	Phe	Phe	Ala	Val	Ser	Lys	Pro	Met
			130					135					140			
	Gly	Thr	Gln	Thr	His	Thr	Met	Ile	Phe	Asp	Asn	Ala	Phe	Asn	Cys	Thr
10145						150						155				160
	Phe	Glu	Tyr	Ile	Ser	Asp	Ala	Phe	Ser	Leu	Asp	Val	Ser	Glu	Lys	Ser
						165				170					175	
	Gly	Asn	Phe	Lys	His	Leu	Arg	Glu	Phe	Val	Phe	Lys	Asn	Lys	Asp	Gly
						180				185					190	
15Phe	Leu	Tyr	Val	Tyr	Lys	Gly	Tyr	Gln	Pro	Ile	Asp	Val	Val	Arg	Asp	
						195			200				205			
	Leu	Pro	Ser	Gly	Phe	Asn	Thr	Leu	Lys	Pro	Ile	Phe	Lys	Leu	Pro	Leu
								215					220			
	Gly	Ile	Asn	Ile	Thr	Asn	Phe	Arg	Ala	Ile	Leu	Thr	Ala	Phe	Ser	Pro
20225						230							235			240
	Ala	Gln	Asp	Ile	Trp	Gly	Thr	Ser	Ala	Ala	Ala	Tyr	Phe	Val	Gly	Tyr
						245				250					255	
	Leu	Lys	Pro	Thr	Thr	Phe	Met	Leu	Lys	Tyr	Asp	Glu	Asn	Gly	Thr	Ile
						260				265					270	
25Thr	Asp	Ala	Val	Asp	Cys	Ser	Gln	Asn	Pro	Leu	Ala	Glu	Leu	Lys	Cys	
						275				280				285		
	Ser	Val	Lys	Ser	Phe	Glu	Ile	Asp	Lys	Gly	Ile	Tyr	Gln	Thr	Ser	Asn
						290			295				300			
	Phe	Arg	Val	Val	Pro	Ser	Gly	Asp	Val	Val	Arg	Phe	Pro	Asn	Ile	Thr
30305						310						315				320
	Asn	Leu	Cys	Pro	Phe	Gly	Glu	Val	Phe	Asn	Ala	Thr	Lys	Phe	Pro	Ser
						325					330				335	
	Val	Tyr	Ala	Trp	Glu	Arg	Lys	Lys	Ile	Ser	Asn	Cys	Val	Ala	Asp	Tyr
						340				345				350		
35Ser	Val	Leu	Tyr	Asn	Ser	Thr	Phe	Phe	Ser	Thr	Phe	Lys	Cys	Tyr	Gly	
						355				360				365		
	Val	Ser	Ala	Thr	Lys	Leu	Asn	Asp	Leu	Cys	Phe	Ser	Asn	Val	Tyr	Ala
						370			375				380			
	Asp	Ser	Phe	Val	Val	Lys	Gly	Asp	Asp	Val	Arg	Gln	Ile	Ala	Pro	Gly
40385						390						395				400
	Gln	Thr	Gly	Val	Ile	Ala	Asp	Tyr	Asn	Tyr	Lys	Leu	Pro	Asp	Asp	Phe
						405					410				415	

34

Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 420 425 430
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 435 440 445
 5Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 450 455 460
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 465 470 475 480
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 10 485 490 495
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 500 505 510
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 515 520 525
 15Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 530 535 540
 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 545 550 555 560
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 20 565 570 575
 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 580 585 590
 Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
 595 600 605
 25Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
 610 615 620
 Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
 625 630 635 640
 His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
 30 645 650 655
 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
 660 665 670
 Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
 675 680 685
 35Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
 690 695 700
 Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
 705 710 715 720
 Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
 40 725 730 735
 Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
 740 745 750

35

Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
 755 760 765
 Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
 770 775 780
 5Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
 785 790 795 800

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<211> 1000

10<212> PRT

<213> SARS coronavirus

<400> 41

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 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 35 40 45
 20Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 50 55 60
 Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 65 70 75 80
 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 25 85 90 95
 Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
 100 105 110
 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 115 120 125
 30Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 130 135 140
 Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 145 150 155 160
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 35 165 170 175
 Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 180 185 190
 Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
 195 200 205
 40Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
 210 215 220

36

Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 225 230 235 240
 Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 245 250 255
 5Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 260 265 270
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 275 280 285
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 10 290 295 300
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 305 310 315 320
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 325 330 335
 15Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 340 345 350
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 355 360 365
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 20 370 375 380
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 385 390 395 400
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 405 410 415
 25Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 420 425 430
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 435 440 445
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 30 450 455 460
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 465 470 475 480
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 485 490 495
 35Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 500 505 510
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 515 520 525
 Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 40 530 535 540
 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 545 550 555 560

37

Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 565 570 575
 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 580 585 590
 5Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
 595 600 605
 Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
 610 615 620
 Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
 10625 630 635 640
 His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
 645 650 655
 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
 660 665 670
 15Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
 675 680 685
 Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
 690 695 700
 Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
 20705 710 715 720
 Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
 725 730 735
 Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
 740 745 750
 25Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
 755 760 765
 Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
 770 775 780
 Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
 30785 790 795 800
 Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
 805 810 815
 Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
 820 825 830
 35Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
 835 840 845
 Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
 850 855 860
 Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
 40865 870 875 880
 Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
 885 890 895

38

Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
 900 905 910
 Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
 915 920 925
 5Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu
 930 935 940
 Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn
 945 950 955 960
 Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp
 10 965 970 975
 Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln
 980 985 990
 Gln Leu Ile Arg Ala Ala Glu Ile
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 Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
 25 20 25 30
 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 35 40 45
 Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 50 55 60
 30Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 65 70 75 80
 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 85 90 95
 Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
 35 100 105 110
 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 115 120 125
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 130 135 140
 40Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 145 150 155 160

39

Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 165 170 175
 Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 180 185 190
 5Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
 195 200 205
 Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
 210 215 220
 Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 10225 230 235 240
 Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 245 250 255
 Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 260 265 270
 15Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 275 280 285
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 290 295 300
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 20305 310 315 320
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 325 330 335
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 340 345 350
 25Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 355 360 365
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 370 375 380
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 30385 390 395 400
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 405 410 415
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 420 425 430
 35Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 435 440 445
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 450 455 460
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 40465 470 475 480
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 485 490 495

40

Val	Val	Leu	Ser	Phe	Glu	Leu	Leu	Asn	Ala	Pro	Ala	Thr	Val	Cys	Gly			
			500					505					510					
Pro	Lys	Leu	Ser	Thr	Asp	Leu	Ile	Lys	Asn	Gln	Cys	Val	Asn	Phe	Asn			
		515					520					525						
5Phe	Asn	Gly	Leu	Thr	Gly	Thr	Gly	Val	Leu	Thr	Pro	Ser	Ser	Lys	Arg			
		530				535					540							
Phe	Gln	Pro	Phe	Gln	Gln	Phe	Gly	Arg	Asp	Val	Ser	Asp	Phe	Thr	Asp			
545					550				555						560			
Ser	Val	Arg	Asp	Pro	Lys	Thr	Ser	Glu	Ile	Leu	Asp	Ile	Ser	Pro	Cys			
10			565					570						575				
Ala	Phe	Gly	Gly	Val	Ser	Val	Ile	Thr	Pro	Gly	Thr	Asn	Ala	Ser	Ser			
			580					585					590					
Glu	Val	Ala	Val	Leu	Tyr	Gln	Asp	Val	Asn	Cys	Thr	Asp	Val	Ser	Thr			
		595				600						605						
15Ala	Ile	His	Ala	Asp	Gln	Leu	Thr	Pro	Ala	Trp	Arg	Ile	Tyr	Ser	Thr			
		610				615					620							
Gly	Asn	Asn	Val	Phe	Gln	Thr	Gln	Ala	Gly	Cys	Leu	Ile	Gly	Ala	Glu			
625					630				635						640			
His	Val	Asp	Thr	Ser	Tyr	Glu	Cys	Asp	Ile	Pro	Ile	Gly	Ala	Gly	Ile			
20			645					650					655					
Cys	Ala	Ser	Tyr	His	Thr	Val	Ser	Leu	Leu	Arg	Ser	Thr	Ser	Gln	Lys			
			660					665					670					
Ser	Ile	Val	Ala	Tyr	Thr	Met	Ser	Leu	Gly	Ala	Asp	Ser	Ser	Ile	Ala			
		675				680							685					
25Tyr	Ser	Asn	Asn	Thr	Ile	Ala	Ile	Pro	Thr	Asn	Phe	Ser	Ile	Ser	Ile			
		690				695					700							
Thr	Thr	Glu	Val	Met	Pro	Val	Ser	Met	Ala	Lys	Thr	Ser	Val	Asp	Cys			
705					710				715						720			
Asn	Met	Tyr	Ile	Cys	Gly	Asp	Ser	Thr	Glu	Cys	Ala	Asn	Leu	Leu	Leu			
30				725				730					735					
Gln	Tyr	Gly	Ser	Phe	Cys	Thr	Gln	Leu	Asn	Arg	Ala	Leu	Ser	Gly	Ile			
			740					745					750					
Ala	Ala	Glu	Gln	Asp	Arg	Asn	Thr	Arg	Glu	Val	Phe	Ala	Gln	Val	Lys			
		755				760							765					
35Gln	Met	Tyr	Lys	Thr	Pro	Thr	Leu	Lys	Tyr	Phe	Gly	Gly	Phe	Asn	Phe			
		770				775							780					
Ser	Gln	Ile	Leu	Pro	Asp	Pro	Leu	Lys	Pro	Thr	Lys	Arg	Ser	Phe	Ile			
785					790					795					800			
Glu	Asp	Leu	Leu	Phe	Asn	Lys	Val	Thr	Leu	Ala	Asp	Ala	Gly	Phe	Met			
40				805				810					815					
Lys	Gln	Tyr	Gly	Glu	Cys	Leu	Gly	Asp	Ile	Asn	Ala	Arg	Asp	Leu	Ile			
			820					825					830					

41

Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
 835 840 845
 Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
 850 855 860
 5Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
 865 870 875 880
 Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
 885 890 895
 Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
 10 900 905 910
 Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
 915 920 925
 Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu
 930 935 940
 15Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn
 945 950 955 960
 Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp
 965 970 975
 Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln
 20 980 985 990
 Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala Ala
 995 1000 1005
 Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val Asp Phe
 1010 1015 1020
 25Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ala Ala Pro His
 1025 1030 1035 1040
 Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ser Gln Glu Arg Asn
 1045 1050 1055
 Phe Thr Thr Ala Pro Ala Ile Cys His Glu Gly Lys Ala Tyr Phe Pro
 30 1060 1065 1070
 Arg Glu Gly Val Phe Val Phe Asn Gly Thr Ser Trp Phe Ile Thr Gln
 1075 1080 1085
 Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr Thr Asp Asn Thr Phe Val
 1090 1095 1100
 35Ser Gly Asn Cys Asp Val Val Ile Gly Ile Ile Asn Asn Thr Val Tyr
 1105 1110 1115 1120
 Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu Asp Lys
 1125 1130 1135
 Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile Ser
 40 1140 1145 1150
 Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu
 1155 1160 1165

42

Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu
 1170 1175 1180

Leu Gly Lys Tyr Glu Gln
 1185 1190

5

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 <212> PRT
 <213> SARS coronavirus

10

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 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 15 20 25 30
 Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 35 40 45
 Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 50 55 60
 20 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 65 70 75 80
 Val Val Arg Gly

25<210> 44

<211> 184
 <212> PRT
 <213> SARS coronavirus

30<400> 44

Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
 1 5 10 15
 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 20 25 30
 35 Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 35 40 45
 Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 50 55 60
 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 40 65 70 75 80
 Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
 85 90 95

43

Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 100 105 110
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 115 120 125
 5Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 130 135 140
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 145 150 155 160
 Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 10 165 170 175
 Phe Leu Tyr Val Tyr Lys Gly Tyr
 180

<210> 45

15<211> 384

<212> PRT

<213> SARS coronavirus

<400> 45

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 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 20 25 30
 Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 25 35 40 45
 Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 50 55 60
 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 65 70 75 80
 30Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
 85 90 95
 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 100 105 110
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 35 115 120 125
 Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 130 135 140
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 145 150 155 160
 40Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 165 170 175

44

Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
 180 185 190
 Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
 195 200 205
 5Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 210 215 220
 Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 225 230 235 240
 Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 10 245 250 255
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 260 265 270
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 275 280 285
 15Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 290 295 300
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 305 310 315 320
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 20 325 330 335
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 340 345 350
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 355 360 365
 25Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 370 375 380

<210> 46

<211> 584

30<212> PRT

<213> SARS coronavirus

<400> 46

Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
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 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 20 25 30
 Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 35 40 45
 40Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 50 55 60

45

Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 65 70 75 80
 Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
 85 90 95
 5Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 100 105 110
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 115 120 125
 Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 10 130 135 140
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 145 150 155 160
 Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 165 170 175
 15Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
 180 185 190
 Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
 195 200 205
 Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 20 210 215 220
 Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 225 230 235 240
 Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 245 250 255
 25Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 260 265 270
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 275 280 285
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 30 290 295 300
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 305 310 315 320
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 325 330 335
 35Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 340 345 350
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 355 360 365
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 40 370 375 380
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 385 390 395 400

46

Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 405 410 415
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 420 425 430
 5Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 435 440 445
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 450 455 460
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 10465 470 475 480
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 485 490 495
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 500 505 510
 15Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 515 520 525
 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 530 535 540
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
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 Glu Val Ala Val Leu Tyr Gln Asp
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 <213> SARS coronavirus

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 35 40 45
 Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 50 55 60
 40Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 65 70 75 80

47

Val	Val	Arg	Gly	Trp	Val	Phe	Gly	Ser	Thr	Met	Asn	Asn	Lys	Ser	Gln
			85						90					95	
Ser	Val	Ile	Ile	Ile	Asn	Asn	Ser	Thr	Asn	Val	Val	Ile	Arg	Ala	Cys
			100					105					110		
5Asn	Phe	Glu	Leu	Cys	Asp	Asn	Pro	Phe	Phe	Ala	Val	Ser	Lys	Pro	Met
			115				120						125		
Gly	Thr	Gln	Thr	His	Thr	Met	Ile	Phe	Asp	Asn	Ala	Phe	Asn	Cys	Thr
			130				135					140			
Phe	Glu	Tyr	Ile	Ser	Asp	Ala	Phe	Ser	Leu	Asp	Val	Ser	Glu	Lys	Ser
10145						150				155					160
Gly	Asn	Phe	Lys	His	Leu	Arg	Glu	Phe	Val	Phe	Lys	Asn	Lys	Asp	Gly
				165					170					175	
Phe	Leu	Tyr	Val	Tyr	Lys	Gly	Tyr	Gln	Pro	Ile	Asp	Val	Val	Arg	Asp
			180					185						190	
15Leu	Pro	Ser	Gly	Phe	Asn	Thr	Leu	Lys	Pro	Ile	Phe	Lys	Leu	Pro	Leu
			195					200					205		
Gly	Ile	Asn	Ile	Thr	Asn	Phe	Arg	Ala	Ile	Leu	Thr	Ala	Phe	Ser	Pro
			210				215					220			
Ala	Gln	Asp	Ile	Trp	Gly	Thr	Ser	Ala	Ala	Ala	Tyr	Phe	Val	Gly	Tyr
20225						230				235					240
Leu	Lys	Pro	Thr	Thr	Phe	Met	Leu	Lys	Tyr	Asp	Glu	Asn	Gly	Thr	Ile
				245					250					255	
Thr	Asp	Ala	Val	Asp	Cys	Ser	Gln	Asn	Pro	Leu	Ala	Glu	Leu	Lys	Cys
			260					265						270	
25Ser	Val	Lys	Ser	Phe	Glu	Ile	Asp	Lys	Gly	Ile	Tyr	Gln	Thr	Ser	Asn
			275					280					285		
Phe	Arg	Val	Val	Pro	Ser	Gly	Asp	Val	Val	Arg	Phe	Pro	Asn	Ile	Thr
			290				295				300				
Asn	Leu	Cys	Pro	Phe	Gly	Glu	Val	Phe	Asn	Ala	Thr	Lys	Phe	Pro	Ser
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Val	Tyr	Ala	Trp	Glu	Arg	Lys	Lys	Ile	Ser	Asn	Cys	Val	Ala	Asp	Tyr
				325					330					335	
Ser	Val	Leu	Tyr	Asn	Ser	Thr	Phe	Phe	Ser	Thr	Phe	Lys	Cys	Tyr	Gly
			340					345					350		
35Val	Ser	Ala	Thr	Lys	Leu	Asn	Asp	Leu	Cys	Phe	Ser	Asn	Val	Tyr	Ala
			355				360						365		
Asp	Ser	Phe	Val	Val	Lys	Gly	Asp	Asp	Val	Arg	Gln	Ile	Ala	Pro	Gly
			370				375					380			
Gln	Thr	Gly	Val	Ile	Ala	Asp	Tyr	Asn	Tyr	Lys	Leu	Pro	Asp	Asp	Phe
40385					390					395					400
Met	Gly	Cys	Val	Leu	Ala	Trp	Asn	Thr	Arg	Asn	Ile	Asp	Ala	Thr	Ser
				405					410					415	

Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 420 425 430
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 435 440 445
 5Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 450 455 460
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 465 470 475 480
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 10 485 490 495
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
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 Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 515 520 525
 15Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 530 535 540
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 545 550 555 560
 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 20 565 570 575
 Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
 580 585 590
 Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
 595 600 605
 25Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
 610 615 620
 His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
 625 630 635 640
 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
 30 645 650 655
 Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
 660 665 670
 Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
 675 680 685
 35Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
 690 695 700
 Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
 705 710 715 720
 Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
 40 725 730 735
 Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
 740 745 750

Gln	Met	Tyr	Lys	Thr	Pro	Thr	Leu	Lys	Tyr	Phe	Gly	Gly	Phe	Asn	Phe	
755					760					765						
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15	20						25				30					
Ser	Asp	Thr	Leu	Tyr	Leu	Thr	Gln	Asp	Leu	Phe	Leu	Pro	Phe	Tyr	Ser	
35						40				45						
Asn	Val	Thr	Gly	Phe	His	Thr	Ile	Asn	His	Thr	Phe	Gly	Asn	Pro	Val	
50						55				60						
20Ile	Pro	Phe	Lys	Asp	Gly	Ile	Tyr	Phe	Ala	Ala	Thr	Glu	Lys	Ser	Asn	
65				70						75				80		
Val	Val	Arg	Gly	Trp	Val	Phe	Gly	Ser	Thr	Met	Asn	Asn	Lys	Ser	Gln	
85						90				95						
Ser	Val	Ile	Ile	Ile	Asn	Asn	Ser	Thr	Asn	Val	Val	Ile	Arg	Ala	Cys	
25	100						105				110					
Asn	Phe	Glu	Leu	Cys	Asp	Asn	Pro	Phe	Phe	Ala	Val	Ser	Lys	Pro	Met	
115						120				125						
Gly	Thr	Gln	Thr	His	Thr	Met	Ile	Phe	Asp	Asn	Ala	Phe	Asn	Cys	Thr	
130						135				140						
30Phe	Glu	Tyr	Ile	Ser	Asp	Ala	Phe	Ser	Leu	Asp	Val	Ser	Glu	Lys	Ser	
145				150						155				160		
Gly	Asn	Phe	Lys	His	Leu	Arg	Glu	Phe	Val	Phe	Lys	Asn	Lys	Asp	Gly	
165						170				175						
Phe	Leu	Tyr	Val	Tyr	Lys	Gly	Tyr	Gln	Pro	Ile	Asp	Val	Val	Arg	Asp	
35	180						185				190					
Leu	Pro	Ser	Gly	Phe	Asn	Thr	Leu	Lys	Pro	Ile	Phe	Lys	Leu	Pro	Leu	
195						200				205						
Gly	Ile	Asn	Ile	Thr	Asn	Phe	Arg	Ala	Ile	Leu	Thr	Ala	Phe	Ser	Pro	
210						215				220						
40Ala	Gln	Asp	Ile	Trp	Gly	Thr	Ser	Ala	Ala	Ala	Tyr	Phe	Val	Gly	Tyr	
225				230						235				240		

50

Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 245 250 255
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 260 265 270
 5Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 275 280 285
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 290 295 300
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 10305 310 315 320
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 325 330 335
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 340 345 350
 15Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 355 360 365
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 370 375 380
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 20385 390 395 400
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 405 410 415
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 420 425 430
 25Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 435 440 445
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 450 455 460
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 30465 470 475 480
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 485 490 495
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 500 505 510
 35Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 515 520 525
 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 530 535 540
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 40545 550 555 560
 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 565 570 575

51

Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
 580 585 590
 Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
 595 600 605
 5Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
 610 615 620
 His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
 625 630 635 640
 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
 10 645 650 655
 Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
 660 665 670
 Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
 675 680 685
 15Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
 690 695 700
 Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
 705 710 715 720
 Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
 20 725 730 735
 Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
 740 745 750
 Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
 755 760 765
 25Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
 770 775 780
 Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
 785 790 795 800
 Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
 30 805 810 815
 Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
 820 825 830
 Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
 835 840 845
 35Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
 850 855 860
 Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
 865 870 875 880
 Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
 40 885 890 895
 Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
 900 905 910

15<213> SARS coronavirus

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				20				25					30			
	Ser	Asp	Thr	Leu	Tyr	Leu	Thr	Gln	Asp	Leu	Phe	Leu	Pro	Phe	Tyr	Ser
				35				40				45				
	Asn	Val	Thr	Gly	Phe	His	Thr	Ile	Asn	His	Thr	Phe	Gly	Asn	Pro	Val
25		50					55					60				
	Ile	Pro	Phe	Lys	Asp	Gly	Ile	Tyr	Phe	Ala	Ala	Thr	Glu	Lys	Ser	Asn
65						70					75					80
	Val	Val	Arg	Gly	Trp	Val	Phe	Gly	Ser	Thr	Met	Asn	Asn	Lys	Ser	Gln
					85					90					95	
30	Ser	Val	Ile	Ile	Ile	Asn	Asn	Ser	Thr	Asn	Val	Val	Ile	Arg	Ala	Cys
					100				105					110		
	Asn	Phe	Glu	Leu	Cys	Asp	Asn	Pro	Phe	Phe	Ala	Val	Ser	Lys	Pro	Met
					115				120				125			
	Gly	Thr	Gln	Thr	His	Thr	Met	Ile	Phe	Asp	Asn	Ala	Phe	Asn	Cys	Thr
35		130					135					140				
	Phe	Glu	Tyr	Ile	Ser	Asp	Ala	Phe	Ser	Leu	Asp	Val	Ser	Glu	Lys	Ser
145						150					155					160
	Gly	Asn	Phe	Lys	His	Leu	Arg	Glu	Phe	Val	Phe	Lys	Asn	Lys	Asp	Gly
					165					170					175	
40	Phe	Leu	Tyr	Val	Tyr	Lys	Gly	Tyr	Gln	Pro	Ile	Asp	Val	Val	Arg	Asp
					180				185					190		

53

Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
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 Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 210 215 220
 5Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 225 230 235 240
 Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 245 250 255
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 10 260 265 270
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 275 280 285
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 290 295 300
 15Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 305 310 315 320
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 325 330 335
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 20 340 345 350
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 355 360 365
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 370 375 380
 25Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 385 390 395 400
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 405 410 415
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 30 420 425 430
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 435 440 445
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 450 455 460
 35Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 465 470 475 480
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 485 490 495
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 40 500 505 510
 Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 515 520 525

54

Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
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 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
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 5Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 565 570 575
 Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
 580 585 590
 Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
 10 595 600 605
 Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
 610 615 620
 His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
 625 630 635 640
 15Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
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 Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
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 Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
 20 675 680 685
 Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
 690 695 700
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 25Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
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 Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
 740 745 750
 Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
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 Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
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 Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
 785 790 795 800
 35Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
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 Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
 820 825 830
 Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
 40 835 840 845
 Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
 850 855 860

55

Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
 865 870 875 880
 Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
 885 890 895
 5 Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
 900 905 910
 Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu
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 Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn
 10 930 935 940
 Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp
 945 950 955 960
 Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln
 965 970 975
 15 Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala Ala
 980 985 990
 Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val Asp Phe
 995 1000 1005
 Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ala Ala Pro His
 20 1010 1015 1020
 Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ser Gln Glu Arg Asn
 1025 1030 1035 1040
 Phe Thr Thr Ala Pro Ala Ile Cys His Glu Gly Lys Ala Tyr Phe Pro
 1045 1050 1055
 25 Arg Glu Gly Val Phe Val Phe Asn Gly Thr Ser Trp Phe Ile Thr Gln
 1060 1065 1070
 Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr Thr Asp Asn Thr Phe Val
 1075 1080 1085
 Ser Gly Asn Cys Asp Val Val Ile Gly Ile Ile Asn Asn Thr Val Tyr
 30 1090 1095 1100
 Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu Asp Lys
 1105 1110 1115 1120
 Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile Ser
 1125 1130 1135
 35 Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu
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 Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu
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 Leu Gly Lys Tyr Glu Gln
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56

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<211> 260

<212> PRT

<213> SARS coronavirus

5

<400> 50

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Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
      35              40              45
Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
      50              55              60
15Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
      65              70              75              80
Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
      85              90              95
Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
20              100              105              110
Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
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Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
      130              135              140
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      145              150              155              160
Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
      165              170              175
Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
30              180              185              190
Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
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Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
      210              215              220
35Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
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Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
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Thr Asp Ala Val
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57

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5

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Asp	Arg	Cys	Thr	Thr	Phe	Asp	Asp	Val	Gln	Ala	Pro	Asn	Tyr	Thr	Gln
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His	Thr	Ser	Ser	Met	Arg	Gly	Val	Tyr	Tyr	Pro	Asp	Glu	Ile	Phe	Arg
10			20				25					30			
Ser	Asp	Thr	Leu	Tyr	Leu	Thr	Gln	Asp	Leu	Phe	Leu	Pro	Phe	Tyr	Ser
		35				40					45				
Asn	Val	Thr	Gly	Phe	His	Thr	Ile	Asn	His	Thr	Phe	Gly	Asn	Pro	Val
	50					55				60					
15Ile	Pro	Phe	Lys	Asp	Gly	Ile	Tyr	Phe	Ala	Ala	Thr	Glu	Lys	Ser	Asn
	65				70				75					80	
Val	Val	Arg	Gly	Trp	Val	Phe	Gly	Ser	Thr	Met	Asn	Asn	Lys	Ser	Gln
			85					90					95		
Ser	Val	Ile	Ile	Ile	Asn	Asn	Ser	Thr	Asn	Val	Val	Ile	Arg	Ala	Cys
20			100					105				110			
Asn	Phe	Glu	Leu	Cys	Asp	Asn	Pro	Phe	Phe	Ala	Val	Ser	Lys	Pro	Met
		115					120				125				
Gly	Thr	Gln	Thr	His	Thr	Met	Ile	Phe	Asp	Asn	Ala	Phe	Asn	Cys	Thr
	130					135					140				
25Phe	Glu	Tyr	Ile	Ser	Asp	Ala	Phe	Ser	Leu	Asp	Val	Ser	Glu	Lys	Ser
	145				150				155					160	
Gly	Asn	Phe	Lys	His	Leu	Arg	Glu	Phe	Val	Phe	Lys	Asn	Lys	Asp	Gly
			165					170				175			
Phe	Leu	Tyr	Val	Tyr	Lys	Gly	Tyr	Gln	Pro	Ile	Asp	Val	Val	Arg	Asp
30			180					185				190			
Leu	Pro	Ser	Gly	Phe	Asn	Thr	Leu	Lys	Pro	Ile	Phe	Lys	Leu	Pro	Leu
		195					200				205				
Gly	Ile	Asn	Ile	Thr	Asn	Phe	Arg	Ala	Ile	Leu	Thr	Ala	Phe	Ser	Pro
	210					215				220					
35Ala	Gln	Asp	Ile	Trp	Gly	Thr	Ser	Ala	Ala	Ala	Tyr	Phe	Val	Gly	Tyr
	225				230				235					240	
Leu	Lys	Pro	Thr	Thr	Phe	Met	Leu	Lys	Tyr	Asp	Glu	Asn	Gly	Thr	Ile
			245					250				255			
Thr	Asp	Ala	Val	Asp	Cys	Ser	Gln	Asn	Pro	Leu	Ala	Glu	Leu	Lys	Cys
40			260					265				270			
Ser	Val	Lys	Ser	Phe	Glu	Ile	Asp	Lys	Gly	Ile	Tyr	Gln	Thr	Ser	Asn
			275					280				285			

58

Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 290 295 300
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 305 310 315 320
 5Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 325 330 335
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 340 345 350
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 10 355 360 365
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 370 375 380
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 385 390 395 400
 15Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 405 410 415
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly
 420 425 430

20<210> 52

<211> 521

<212> PRT

<213> SARS coronavirus

25<400> 52

Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
 1 5 10 15
 His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
 20 25 30
 30Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
 35 40 45
 Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
 50 55 60
 Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
 3565 70 75 80
 Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
 85 90 95
 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
 100 105 110
 40Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
 115 120 125

59

Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
 130 135 140
 Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
 145 150 155 160
 5Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
 165 170 175
 Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
 180 185 190
 Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
 10 195 200 205
 Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
 210 215 220
 Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
 225 230 235 240
 15Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
 245 250 255
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 260 265 270
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 20 275 280 285
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 290 295 300
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 305 310 315 320
 25Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 325 330 335
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 340 345 350
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 30 355 360 365
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 370 375 380
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 385 390 395 400
 35Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 405 410 415
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 420 425 430
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 40 435 440 445
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 450 455 460

60

Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 465 470 475 480
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 485 490 495
 5Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 500 505 510
 Phe Asn Gly Leu Thr Gly Thr Gly Val
 515 520

10<210> 53

<211> 777

<212> PRT

<213> Artificial Sequence

15<220>

<223> Synthetic sequence of amino acids 17-757 of SEQ ID NO:1 plus an
 N-terminal mouse K chain leader sequence and a C-terminal myc
 epitope and a polyhistidine tag

20<400> 53

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15
 Gly Ser Thr Gly Asp Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala
 20 25 30
 25Pro Asn Tyr Thr Gln His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro
 35 40 45
 Asp Glu Ile Phe Arg Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe
 50 55 60
 Leu Pro Phe Tyr Ser Asn Val Thr Gly Phe His Thr Ile Asn His Thr
 3065 70 75 80
 Phe Gly Asn Pro Val Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala
 85 90 95
 Thr Glu Lys Ser Asn Val Val Arg Gly Trp Val Phe Gly Ser Thr Met
 100 105 110
 35Asn Asn Lys Ser Gln Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val
 115 120 125
 Val Ile Arg Ala Cys Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala
 130 135 140
 Val Ser Lys Pro Met Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn
 40145 150 155 160
 Ala Phe Asn Cys Thr Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp
 165 170 175

Val	Ser	Glu	Lys	Ser	Gly	Asn	Phe	Lys	His	Leu	Arg	Glu	Phe	Val	Phe
			180						185			190			
Lys	Asn	Lys	Asp	Gly	Phe	Leu	Tyr	Val	Tyr	Lys	Gly	Tyr	Gln	Pro	Ile
			195						200			205			
5Asp	Val	Val	Arg	Asp	Leu	Pro	Ser	Gly	Phe	Asn	Thr	Leu	Lys	Pro	Ile
			210						215			220			
Phe	Lys	Leu	Pro	Leu	Gly	Ile	Asn	Ile	Thr	Asn	Phe	Arg	Ala	Ile	Leu
225				230						235			240		
Thr	Ala	Phe	Ser	Pro	Ala	Gln	Asp	Ile	Trp	Gly	Thr	Ser	Ala	Ala	Ala
10				245						250			255		
Tyr	Phe	Val	Gly	Tyr	Leu	Lys	Pro	Thr	Thr	Phe	Met	Leu	Lys	Tyr	Asp
			260						265			270			
Glu	Asn	Gly	Thr	Ile	Thr	Asp	Ala	Val	Asp	Cys	Ser	Gln	Asn	Pro	Leu
			275						280			285			
15Ala	Glu	Leu	Lys	Cys	Ser	Val	Lys	Ser	Phe	Glu	Ile	Asp	Lys	Gly	Ile
			290						295			300			
Tyr	Gln	Thr	Ser	Asn	Phe	Arg	Val	Val	Pro	Ser	Gly	Asp	Val	Val	Arg
305				310						315			320		
Phe	Pro	Asn	Ile	Thr	Asn	Leu	Cys	Pro	Phe	Gly	Glu	Val	Phe	Asn	Ala
20				325						330			335		
Thr	Lys	Phe	Pro	Ser	Val	Tyr	Ala	Trp	Glu	Arg	Lys	Lys	Ile	Ser	Asn
			340						345			350			
Cys	Val	Ala	Asp	Tyr	Ser	Val	Leu	Tyr	Asn	Ser	Thr	Phe	Phe	Ser	Thr
			355						360			365			
25Phe	Lys	Cys	Tyr	Gly	Val	Ser	Ala	Thr	Lys	Leu	Asn	Asp	Leu	Cys	Phe
			370						375			380			
Ser	Asn	Val	Tyr	Ala	Asp	Ser	Phe	Val	Val	Lys	Gly	Asp	Asp	Val	Arg
385				390						395			400		
Gln	Ile	Ala	Pro	Gly	Gln	Thr	Gly	Val	Ile	Ala	Asp	Tyr	Asn	Tyr	Lys
30				405						410			415		
Leu	Pro	Asp	Asp	Phe	Met	Gly	Cys	Val	Leu	Ala	Trp	Asn	Thr	Arg	Asn
			420						425			430			
Ile	Asp	Ala	Thr	Ser	Thr	Gly	Asn	Tyr	Asn	Tyr	Lys	Tyr	Arg	Tyr	Leu
			435						440			445			
35Arg	His	Gly	Lys	Leu	Arg	Pro	Phe	Glu	Arg	Asp	Ile	Ser	Asn	Val	Pro
			450						455			460			
Phe	Ser	Pro	Asp	Gly	Lys	Pro	Cys	Thr	Pro	Pro	Ala	Leu	Asn	Cys	Tyr
465				470						475			480		
Trp	Pro	Leu	Asn	Asp	Tyr	Gly	Phe	Tyr	Thr	Thr	Thr	Gly	Ile	Gly	Tyr
40				485						490			495		
Gln	Pro	Tyr	Arg	Val	Val	Val	Leu	Ser	Phe	Glu	Leu	Leu	Asn	Ala	Pro
			500						505			510			

62

Ala Thr Val Cys Gly Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln
 515 520 525
 Cys Val Asn Phe Asn Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr
 530 535 540
 5Pro Ser Ser Lys Arg Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val
 545 550 555 560
 Ser Asp Phe Thr Asp Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu
 565 570 575
 Asp Ile Ser Pro Cys Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly
 10 580 585 590
 Thr Asn Ala Ser Ser Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys
 595 600 605
 Thr Asp Val Ser Thr Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp
 610 615 620
 15Arg Ile Tyr Ser Thr Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys
 625 630 635 640
 Leu Ile Gly Ala Glu His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro
 645 650 655
 Ile Gly Ala Gly Ile Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg
 20 660 665 670
 Ser Thr Ser Gln Lys Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala
 675 680 685
 Asp Ser Ser Ile Ala Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn
 690 695 700
 25Phe Ser Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser Met Ala Lys
 705 710 715 720
 Thr Ser Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys
 725 730 735
 Ala Asn Leu Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg
 30 740 745 750
 Ala Leu Ser Gly Ile Ala Ala Glu Gln Glu Gln Lys Leu Ile Ser Glu
 755 760 765
 Glu Asp Leu His His His His His His
 770 775
 35
 <210> 54
 <211> 297
 <212> PRT
 <213> Artificial Sequence
 40
 <220>
 <223> Synthetic sequence of amino acids 17-276 of SEQ ID NO:1 plus an

63

N-terminal mouse K chain leader sequence and a C-terminal myc epitope and a polyhistidine tag

```

<400> 54
5Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1          5          10          15
Gly Ser Thr Gly Asp Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala
  20          25          30
Pro Asn Tyr Thr Gln His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro
10      35          40          45
Asp Glu Ile Phe Arg Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe
  50          55          60
Leu Pro Phe Tyr Ser Asn Val Thr Gly Phe His Thr Ile Asn His Thr
65      70          75          80
15Phe Gly Asn Pro Val Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala
      85          90          95
Thr Glu Lys Ser Asn Val Val Arg Gly Trp Val Phe Gly Ser Thr Met
      100          105          110
Asn Asn Lys Ser Gln Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val
20      115          120          125
Val Ile Arg Ala Cys Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala
      130          135          140
Val Ser Lys Pro Met Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn
145      150          155          160
25Ala Phe Asn Cys Thr Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp
      165          170          175
Val Ser Glu Lys Ser Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe
      180          185          190
Lys Asn Lys Asp Gly Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile
30      195          200          205
Asp Val Val Arg Asp Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile
      210          215          220
Phe Lys Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu
225      230          235          240
35Thr Ala Phe Ser Pro Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala
      245          250          255
Tyr Phe Val Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp
      260          265          270
Glu Asn Gly Thr Ile Thr Asp Ala Val Glu Gln Lys Leu Ile Ser Glu
40      275          280          285
Glu Asp Leu His His His His His His
      290          295

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64

<210> 55

<211> 558

<212> PRT

<213> Artificial Sequence

5

<220>

<223> A synthetic sequence of amino acids 17-537 of SEQ ID NO:1 plus an N-terminal mouse K chain leader sequence and a C-terminal myc epitope and a polyhistidine tag

10

<400> 55

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1              5              10              15
Gly Ser Thr Gly Asp Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala
15              20              25              30
Pro Asn Tyr Thr Gln His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro
              35              40              45
Asp Glu Ile Phe Arg Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe
 50              55              60
20Leu Pro Phe Tyr Ser Asn Val Thr Gly Phe His Thr Ile Asn His Thr
 65              70              75              80
Phe Gly Asn Pro Val Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala
              85              90              95
Thr Glu Lys Ser Asn Val Val Arg Gly Trp Val Phe Gly Ser Thr Met
25              100             105             110
Asn Asn Lys Ser Gln Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val
              115             120             125
Val Ile Arg Ala Cys Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala
              130             135             140
30Val Ser Lys Pro Met Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn
 145             150             155             160
Ala Phe Asn Cys Thr Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp
              165             170             175
Val Ser Glu Lys Ser Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe
35              180             185             190
Lys Asn Lys Asp Gly Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile
              195             200             205
Asp Val Val Arg Asp Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile
              210             215             220
40Phe Lys Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu
 225             230             235             240

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65

Thr Ala Phe Ser Pro Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala
 245 250 255
 Tyr Phe Val Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp
 260 265 270
 5Glu Asn Gly Thr Ile Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu
 275 280 285
 Ala Glu Leu Lys Cys Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile
 290 295 300
 Tyr Gln Thr Ser Asn Phe Arg Val Val Pro Ser Gly Asp Val Val Arg
 10305 310 315 320
 Phe Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala
 325 330 335
 Thr Lys Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn
 340 345 350
 15Cys Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr
 355 360 365
 Phe Lys Cys Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe
 370 375 380
 Ser Asn Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg
 20385 390 395 400
 Gln Ile Ala Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys
 405 410 415
 Leu Pro Asp Asp Phe Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn
 420 425 430
 25Ile Asp Ala Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu
 435 440 445
 Arg His Gly Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro
 450 455 460
 Phe Ser Pro Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr
 30465 470 475 480
 Trp Pro Leu Asn Asp Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr
 485 490 495
 Gln Pro Tyr Arg Val Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro
 500 505 510
 35Ala Thr Val Cys Gly Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln
 515 520 525
 Cys Val Asn Phe Asn Phe Asn Gly Leu Thr Gly Thr Gly Val Glu Gln
 530 535 540
 Lys Leu Ile Ser Glu Glu Asp Leu His His His His His His
 40545 550 555

66

<210> 56

<211> 739

<212> PRT

<213> Artificial Sequence

5

<220>

<223> A synthetic sequence of amino acids 17-756 of SEQ ID NO:1
without a signal peptide at the N-terminus

10<400> 56

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Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
 1           5           10           15
His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
          20           25           30
15Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
          35           40           45
Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
          50           55           60
Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
2065          70           75           80
Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
          85           90           95
Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
          100          105          110
25Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
          115          120          125
Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
          130          135          140
Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
30145          150          155          160
Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
          165          170          175
Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
          180          185          190
35Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
          195          200          205
Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
          210          215          220
Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
40225          230          235          240
Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
          245          250          255

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Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
 260 265 270
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
 275 280 285
 5Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
 290 295 300
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 305 310 315 320
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 10 325 330 335
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 340 345 350
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 355 360 365
 15Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 370 375 380
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 385 390 395 400
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 20 405 410 415
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 420 425 430
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 435 440 445
 25Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 450 455 460
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 465 470 475 480
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 30 485 490 495
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 500 505 510
 Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 515 520 525
 35Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 530 535 540
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 545 550 555 560
 Ala Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 40 565 570 575
 Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
 580 585 590

68

Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
 595 600 605
 Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
 610 615 620
 5His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
 625 630 635 640
 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
 645 650 655
 Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
 10 660 665 670
 Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
 675 680 685
 Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
 690 695 700
 15Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
 705 710 715 720
 Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
 725 730 735
 Ala Ala Glu

20

<210> 57

<211> 265

<212> PRT

25<213> Artificial Sequence

<220>

<223> A synthetic sequence of amino acids 272-537 of SEQ ID NO:1

30<400> 57

Ile Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys
 1 5 10 15
 Cys Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser
 20 25 30
 35Asn Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Asn Ile Thr
 35 40 45
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
 50 55 60
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
 4065 70 75 80
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
 85 90 95

69

Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
 100 105 110
 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
 115 120 125
 5Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
 130 135 140
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
 145 150 155 160
 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 10 165 170 175
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 180 185 190
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 195 200 205
 15Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 210 215 220
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 225 230 235 240
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 20 245 250 255
 Phe Asn Gly Leu Thr Gly Thr Gly Val
 260 265

<210> 58

25<211> 17

<212> PRT

<213> SARS coronavirus

<400> 58

30Asp Val Gln Ala Pro Asn Tyr Thr Gln His Thr Ser Ser Met Arg Gly
 1 5 10 15
 Cys

35<210> 59

<211> 15

<212> PRT

<213> SARS coronavirus

40<400> 59

Pro Ser Ser Lys Arg Phe Gln Pro Gln Gln Phe Gly Arg Asp Cys
 1 5 10 15

70

<210> 60

<211> 16

<212> PRT

<213> SARS coronavirus

5

<400> 60

Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
 1 5 10 15

10<210> 61

<211> 235

<212> PRT

<213> Artificial Sequence

15<220>

<223> A synthetic sequence of amino acids 303-537 of SEQ ID NO:1
 containing the receptor binding domain

<400> 61

20Ser Asn Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn
 1 5 10 15
 Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe
 20 25 30
 Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala
 25 35 40 45
 Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys
 50 55 60
 Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val
 65 70 75 80
 30Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala
 85 90 95
 Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp
 100 105 110
 Asp Phe Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala
 35 115 120 125
 Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly
 130 135 140
 Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro
 145 150 155 160
 40Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu
 165 170 175

71

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Asn Asp Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr
      180                      185                      190
Arg Val Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val
      195                      200                      205
5Cys Gly Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn
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Phe Asn Phe Asn Gly Leu Thr Gly Thr Gly Val
      225                      230                      235

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   1              5              10              15
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      20              25              30
Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys
25      35              40              45
Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val
      50              55              60
Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala
      65              70              75              80
30Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp
      85              90              95
Asp Phe Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala
      100             105             110
Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly
35      115             120             125
Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro
      130             135             140
Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu
      145             150             155             160
40Asn Asp Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr
      165             170             175

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Arg Val Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val
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 Cys Gly Pro Lys Leu Ser Thr
 195
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 containing the receptor binding domain
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 20 25 30
 20Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys
 35 40 45
 Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val
 50 55 60
 Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala
 2565 70 75 80
 Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp
 85 90 95
 Asp Phe Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala
 100 105 110
 30Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly
 115 120 125
 Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro
 130 135 140
 Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu
 35145 150 155 160
 Asn Asp Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr
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 40Cys Gly Pro Lys Leu Ser Thr Asp
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73

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<212> DNA

15<213> Artificial Sequence

<220>

<223> A synthetic primer

20<400> 65

gatcggggccc gacacactgg ttc

23

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/023345

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/165 A61K39/215 A61K39/42 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE, MEDLINE, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 23 April 2003 (2003-04-23), "SARS coronavirus urbani, complete genome." XP002304795 retrieved from EBI Database accession no. AY278741 abstract	1-84
X	-& ROTA P A ET AL: "Characterization of a novel coronavirus associated with severe acute respiratory syndrome" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 300, no. 5624, 30 May 2003 (2003-05-30), pages 1394-1399, XP002269482 ISSN: 0036-8075 the whole document	1-84

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

10 November 2004

Date of mailing of the international search report

26/11/2004

Name and mailing address of the ISA

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Grötzinger, T

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/US2004/023345

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 15 April 2003 (2003-04-15), "SARS coronavirus TOR2, complete genome." XP002304796 retrieved from EBI Database accession no. AY274119 abstract	1-84
X	-& MARRA M A ET AL: "The genome sequence of the SARS-associated coronavirus" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 300, no. 5624, 30 May 2003 (2003-05-30), pages 1399-1404, XP002269483 ISSN: 0036-8075 the whole document	1-84
X	WO 93/23421 A (SMITHKLINE BEECHAM CORP ; JONES ELAINE V (US); KLEPFER SHARON (US); MI) 25 November 1993 (1993-11-25) page 3, line 24 - line 31 page 4, line 6 - line 30	39,40, 44-48, 59-61, 67-69
P,X	DATABASE EMBL 30 November 2003 (2003-11-30), "SARS coronavirus CUHK-A601, complete genome." XP002304797 retrieved from EBI Database accession no. AY345986 abstract	1-84
P,X	-& CHIM S ET AL: "Genomic characterisation of the severe acute respiratory syndrome coronavirus of Amoy Gardens outbreak in Hong Kong" LANCET THE, LANCET LIMITED. LONDON, GB, vol. 362, no. 9398, 29 November 2003 (2003-11-29), pages 1807-1808, XP004476558 ISSN: 0140-6736 the whole document	1-84
P,X	DATABASE EMBL 7 January 2004 (2004-01-07), "SARS coronavirus TW6, complete genome." XP002304798 retrieved from EBI Database accession no. AY502929 abstract -/--	1-84

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US2004/023345

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>-& YEH SHIOU-HWEI ET AL: "Characterization of severe acute respiratory syndrome coronavirus genomes in Taiwan: Molecular epidemiology and genome evolution." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 101, no. 8, 24 February 2004 (2004-02-24), pages 2542-2547, XP002304793 ISSN: 0027-8424 the whole document</p>	1-84
P,X	<p>DATABASE EMBL 28 February 2004 (2004-02-28), "SARS coronavirus TW-GD5 isolate TW-GD5_SC22-23 replicase 1B and spike glycoprotein genes, partial cds." XP002304799 retrieved from EBI Database accession no. AY451903 abstract</p>	1,4-20, 24-30, 37-48, 67-69, 73,74
P,X	<p>YANG ZHI-YONG ET AL: "A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice" NATURE (LONDON), vol. 428, no. 6982, 1 April 2004 (2004-04-01), pages 561-564, XP002304794 ISSN: 0028-0836 abstract page 563, right-hand column, section "Immunogen and plasmid construction"</p>	1-84

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2004/023345

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing
 - ☒ contained in the international application as filed
 - ☒ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/023345

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 59, 62, 66, 67, as well as the dependent claims are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/023345

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9323421	A	25-11-1993	
		AU 678970 B2	19-06-1997
		AU 4240493 A	13-12-1993
		AU 678971 B2	19-06-1997
		AU 4241093 A	13-12-1993
		CA 2134898 A1	25-11-1993
		CA 2135201 A1	25-11-1993
		EP 0640096 A1	01-03-1995
		EP 0640097 A1	01-03-1995
		JP 7508176 T	14-09-1995
		JP 8501931 T	05-03-1996
		WO 9323421 A1	25-11-1993
		WO 9323422 A1	25-11-1993